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THE RELATION BETWEEN PURE AND APPLIED BIOLOGY

BY PROFESSOR E. B. POULTON, M.A., D.Sc., LL.D., F.R.S.

BEING THE ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION, DELIVERED JANUARY 18TH, 1924.

AT the end of my term of office it is a pleasing duty to thank the officers of the Association who have made my work so light, the readers of papers from whom we have learned so much of interest and importance, and those who have contributed to the discussions which have given an added vitality to our meetings. It has been most inspiring to see such numbers of students coming together month after month to listen and I have no doubt to criticise; and this in the early afternoon, a time which for many is held to be sacred to the cult of the open air.

I have all through looked forward to the pleasure and privilege of thanking those who have made my term of office so easy and so interesting, and of expressing the confident hope that the same kind help would be extended to my successor. But when it comes to a Presidential address, that is a different matter. An address if required is a chief, probably the chief, responsibility of the position. I am a very busy man and the work seems to increase as I become older. Furthermore I have had to deliver many addresses. It is a good thing that, from time to time, a man should be induced to look at his subject from a new point of view, and I do not agree with an opinion recently expressed by a senior member of a scientific society, as the anniversary meeting was approaching—that "the whole thing is a waste of time: the President doesn't want to give an address and nobody wants to listen to it!"

A Presidential address is I believe a good thing, but a man should not be expected to give many. Suitable subjects are not very numerous, and, if treated seriously, all of them demand much time and thought. A man owes it to himself, or as I prefer to say, to those who look to him for guidance, not to spend too much time in the preparation of addresses on the more general aspects of his subject. Therefore, when honoured with the invitation, I asked whether an address was necessary and received the assurance that it was not required. I hope that this will

continue to be your tradition. We have many scientific societies in this country and your Presidents will often be men who have previously served elsewhere and some of them may find a real difficulty in again accepting a position which carries this responsibility.

In spite of the assurance given to me I am delivering a brief address in compliance with the wishes of our late Secretary, Dr W. B. Brierley, to whose efficiency and energy we owe so much. He was so persuasive that I felt compelled to compromise and agree to do as he wished, not however twice, but at the end of my term of office. It is the kind of thing one undertakes to do a year-and-a-half ahead—a distant future when the pressure of work will surely be relieved! That is the dream, but presently comes the awakening. It seems to be a law of human nature that as the fatal day approaches and time for thought is urgently required, work of all kinds, work which cannot be put off, steadily accumulates. Therefore today I can only offer you a spoken and not a written address. I hope however that this is not all loss, and that you will agree that speech face to face has some advantages over the more rounded written sentences more or less hidden behind a paper screen.

The revenge I might have taken is denied me. If Dr Brierley had remained in office I might perhaps have followed the example of an eminent President of a scientific society and have written a very polite letter to the Secretary asking him to give me his kind help by preparing notes on the recent advances in our science, and when the notes were received, I might have acted as he did and returned them to the Secretary with the most grateful thanks but asking if he would add to my indebtedness by throwing them into the form of an address!

It is of interest to compare for a moment Applied Biology with Applied Physics and Chemistry. Our friends sometimes take a rather superior tone in comparing their sciences with ours. I remember a Physicist friend who, when it was maintained that it would be useful to have some common term to include Physics and Chemistry just as Biology includes both Zoology and Botany, replied at once "Oh! but we have such a term—Science!" But there is reason to fear that the share in science which my friend allotted to Chemistry was very different from that which he claimed for his own subject. It was a haughty Physicist who defined Chemistry as "The dirty part of Physics and the clean part of Biology!"

In Applied Science, however, Biology has a great advantage over Physics and Chemistry—an advantage which is especially great in the

department of learning with which this Association deals. Every problem concerned with living beings compels the student to search in many directions, to seek all possible help in the widest field, and generally to invoke the assistance of the haughty sciences themselves. The simplest living being is infinitely complex and enters into an infinitely complex indirect relationship with the world around it. The attempt to unravel a part of this entangled web is not only broadening and strengthening to the intellectual faculties but also to the social side of human nature. On other occasions I have dwelt upon the broadening influence of an intelligently directed specialisation, but this aspect is so often neglected that I do not hesitate to speak of it again. The infinitely complex problem set before the student of Applied Biology compels him to seek help from other specialists. His own work shows him the uselessness of an opinion founded on superficial study, and he realises that the rapid glance of one who knows is generally worth far more than a prolonged examination by one who is without an intimate knowledge of the subject. He is thus led to pursue to the uttermost his own researches in his own part of the great field without any inclination to encroach on the sections where others are at work—to give help and to receive help, to take a keen and friendly interest in the investigations going on around him and to welcome the same interest in his own.

Specialisation pursued in this spirit, so far from being narrow and narrowing, is broadening as an education and broadening as a social force; for it leads to mutual appreciation instead of mutual jealousy. It is a spirit very different from that of a not distant past symbolised by Professor Ptthmllnsprts in *The Water-Babies*. Only recently I have come to realise, thanks to Stephen Paget in *I have reason to believe*, that the weird name is "Put them all in spirits" with the vowels left out! Well, you remember that Professor Ptthmllnsprts was said to be like a cock-robin, because "when anyone else found a curious worm, he would hop round them, and peck them, and set up his tail, and bristle up his feathers, just as a cock-robin would; and declare that he found the worm first; and that it was his worm; and, if not, that then it was not a worm at all."

But even in those years of fierce rivalries which seem so far off and are really so near, we find the highest of all examples of mutual sympathy and appreciation in the two great men who, on opposite sides of the world, hit upon the same discovery. On July 1, 1908, the Linnean Society celebrated the fiftieth anniversary of the announcement of the theory of natural selection in the Darwin-Wallace Essay. Darwin and

Sir Charles Lyell had been dead many years but the other chief actors, Alfred Russel Wallace and Sir Joseph Hooker, were present and spoke to us. And the essential message conveyed by Wallace's speech on that great occasion was a protest against the too great credit which he had been given for his part in the discovery. Of Darwin's part in it he said—"If the persuasion of his friends had prevailed with him, and he had published his theory, after 10 years'—15 years'—or even 18 years' elaboration of it—*I* should have had no part in it whatever, and *he* would have been at once recognised, and should be ever recognised, as the sole and undisputed discoverer and patient investigator of the great law of 'Natural Selection' in all its far-reaching consequences."

It may be fairly claimed that the grip of the profession is less constraining in Applied Biology than in Applied Physics and Chemistry. The notice "No road" is far less likely to be displayed over against some fascinating side-path, because in Biology a side-path so often leads to the goal.

Leaving the application on one side for the moment, it is well to realise the motive which impels to research. When you get down to bedrock you will find that the true inducement is the gratification of the instinct of discovery, indulgence, in fact, in a glorified curiosity. I remember the amusing words in which this conclusion was enforced by Michael Foster. "It was by curiosity" he said "that our first parents lost the Garden of Eden, but by transmitting to us that same curiosity they built for us a golden bridge by which we may re-enter Paradise." And surely the glorious moment when scientific curiosity is satisfied takes us into an earthly Paradise if anything can.

The true motive-power of research is stated by Darwin in a letter to Henslow who had maintained that "However delightful any scientific pursuit may be, yet, if it should be wholly unapplied, it is of no more use than building castles in the air." Darwin in reply pointed to the practical uses which came later but could never have come except with the aid of scientific work conducted solely for its own sake. And he continued: "For myself I would, however, take higher ground, for I believe there exists, and I feel within me, an instinct for truth, or knowledge or discovery, of something of the same nature as the instinct of virtue, and that our having such an instinct is reason enough for scientific researches without any practical results ever ensuing from them."[1]

I do not think that Darwin's argument needs enforcing at the present day, for I believe that everyone will agree with it. You will observe that

[1] *More Letters*, I, 61.

there is nothing in his words to discourage applied science, provided that its problems are approached in the spirit of which he wrote. And the success which has attended these enquiries is in itself sufficient evidence that they have been undertaken in this spirit. The immense increase in our knowledge of detailed life-histories and the bionomic relations of animals and plants which we owe to the researches in Economic Biology at Washington, in Hawaii, and in this country at Rothamsted and under the guidance of the Imperial Bureau of Entomology and that of Mycology—all this has meant the love of knowledge for its own sake and, without this inspiration, could not have been achieved. If immediate practical benefits result so much the better; if not, the outcome is still as it is in other scientific researches, increase in the mass of human knowledge, out of which practical benefits are certain to come in the end.

Any man who has had much to do with scientific students will have realised that many of them—a large proportion happily—are full of scientific curiosity—the love and the longing for discovery—and that they are eager to catch at any suggestion made by one older and more experienced. The suggestions for research in Economic Biology are everywhere around us appealing to the same spirit as that in which the young student begins his first investigation for the love and the glory of discovery alone. If they are not accepted in this spirit they had better not be undertaken at all. But I have no fears. As I have already indicated, the results attained speak for themselves, proclaiming that the work has been done in the spirit of all scientific enquiry, in the intense desire to find out, to know why—the motive cause of all discovery and advance.

Before I conclude there are a few general considerations which I should wish to bring before those of you who are looking forward to a life devoted to the problems of Economic Biology.

The Differences between Island and Continental Areas. The attempt to deal with some economic pest is far less difficult on an island than on the mainland. On the smaller area, with its far simpler web of organic life, there is a good chance of exterminating the evil altogether or of reducing it to comparative harmlessness by the introduction of its natural enemies. Of these some, also attacking other species, may find in the limited fauna little or no food except that provided by the injurious species and will then threaten it, and finally themselves, with extermination. Such a result is far less likely on a continental area, and if it should occur, will only be local and the pest can later on re-invade the locality from the surrounding districts. Again, the simpler environment,

inorganic as well as organic, may itself solve or help to solve the problem without any human effort. Storms and other climatic disturbances will tend to produce far greater destruction of life on islands. In a recent attempt to investigate the invasion of the Fijian Islands, and to a less extent other Pacific groups, by certain butterflies and the changes which have taken place in the new home, I have been much struck with the fact that the only evidences of evolution are very modern and superficial and that nothing older has been preserved. There is not only the possibility of hurricanes of exceptional force but of an enemy such as a bird increasing in numbers and entirely destroying some species which it normally only keeps in check. Or again an intruder may itself increase progressively and destroy its own food-plant. All such effects, probable enough on islands, are far less likely to happen over large continental areas.

Unchecked Variation. Whatever the problem before the scientific enquirer he will do well to keep a sharp look out for incidental results of the deepest interest which may often be gained by the way without interrupting the main current of his work or may often-times divert it into a more fruitful channel. The exciting subject of variation is one upon which the Economic Biologist is especially likely to throw light. Under ordinary conditions, with reproduction so far balanced by extermination that the average numbers of a species remain constant, there is reason to believe that variation is held in check by selection. But now and then a time will come—a time in which the help of the Economic Biologist is likely to be required—when a species gets the better of the forces which normally hold it in check, and increases beyond all bounds. The proportionate amount of variation in these temporarily well-nigh selection-free periods is a fascinating subject for study.

The Ebb and Flow of Evolution. The Economic Biologist, like every student of living nature, is sure to consider the bearing of his work upon evolutionary theories and it is therefore well to direct attention to an aspect of evolution which is sometimes neglected. Evolution does not take and probably has never taken a road that leads continuously upward, but one of endless ups and downs, only to be recognised as an advance when averaged over a considerable distance. The upward movement of a single undulation, to be followed at once by an equal or perhaps an even greater descent, is liable to be mistaken for true evolutionary progress. The possibility of such an erroneous interpretation has been forced upon me in studying the changes in the colours and patterns of insects. Colours and patterns were of course evolved from a previous

condition of insect life in which they did not exist, and it is tempting to look upon the changes which a pattern undergoes in a series of allied races as part of the great onward movement of evolutionary history. But further investigation shows that the patterns, once formed, have been subject to constant fluctuation, increasing complexity succeeded by increasing simplicity and this again by complexity in endless succession. I do not doubt that the same ebb and flow is true of other aspects of organic nature where it is less easily detected and therefore all the more likely to suggest a fascinating but erroneous interpretation.

THE CROWN-GALL DISEASE OF NURSERY STOCKS

I. FIELD OBSERVATIONS ON APPLE STOCKS

By H. WORMALD and N. H. GRUBB.
(*Horticultural Research Station, East Malling, Kent.*)

(With Plates VIII and IX.)

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I. Introduction.

Tumourous outgrowths in which there are no obvious parasites are of frequent occurrence on cultivated plants and trees, and their cause was for many years a subject for much speculation among growers and plant pathologists. The earlier observers attributed the disease to mechanica injuries or to frosts. Various organisms have also been suggested from time to time as the causal agents, without, however, until comparatively recently, any convincing proof being put forward of their parasitic nature.

About 1890 the galls on fruit trees began to receive great attention in America, where the disease came to be known as "Crown-Gall," because the galls usually arose on the "crown" or "collar" of the trees, at or near the ground level. In 1900 Toumey(12) put forward the view that

a myxomycete, *Dendrophagus globosus*, which he found in galls on almond trees, was the cause of the abnormal outgrowths. This theory was not generally accepted and a few years later the subject was re-opened at the United States Bureau of Plant Industry at Washington, where Dr Erwin F. Smith and his colleagues (9) were able to prove by numerous experiments that tumours of the Crown Gall type could readily be reproduced by inoculating plants with pure cultures of an organism isolated from naturally produced galls. This gall-inducing organism they named *Bacterium tumefaciens* Smith and Townsend (8).

Numerous papers have been published by American investigators treating chiefly of various aspects of crown-gall which are out of the scope of the present article. The occurrence of galls on fruit trees has been especially studied by Hedgcock (4, 5), who issued a series of pamphlets on the subject from 1905 to 1911; his Bulletin on "Field Studies of the Crown-Gall and Hairy-Root of the Apple Tree" should be consulted for references to work done on fruit tree galls previous to 1911 and for a description of crown-gall as affecting apple stocks in America. A general account of crown-gall and the experimental work connected with recent investigations will be found in Erwin F. Smith's book (11) *An Introduction to Bacterial Diseases of Plants*. Certain interesting facts relating to galls induced by artificial inoculations, carried out in this country on chrysanthemums and tobacco plants, are discussed in a recent paper by Robinson and Walkden (7).

The interest of the present writers was drawn to crown-gall by reason of the fact that some of the types of apple stocks propagated from layers, which are being raised at East Malling Fruit Research Station, proved to be very susceptible to a disease which apparently was crown-gall, and it was decided to make a study of the disease with special reference to its occurrence on those stocks.

On the Apple Stocks at East Malling it was first noticed about six years ago when it was found on old stools of an unknown variety of "Paradise" stock (referred to here as Type VII), and on trees worked on this stock, but it had previously been observed on the roots of certain ornamental species of *Pyrus* which had been obtained from the Continent. A trace at least of the disease has been found, after close examination, on the subterranean parts of every variety of layered apple stocks so far grown at East Malling, though many of the varieties seem to be moderately resistant when compared with Type VII, which still remains the most susceptible of all.

During the course of the investigation some attention has been given

to galls occurring on other hosts and, in addition to those occurring on layered apple stocks, galls, which from their general structure and the absence of any obvious parasites appear to be of the crown-gall type, have been found at East Malling on seedling apple trees, layered pear stocks and layered hawthorn stocks; similar galls on crab stocks, young pear trees, plum trees, rose (particularly Manetti) stocks, raspberry, loganberry, mangel, beetroot, melon, hop and hollyhock have been sent to us from various parts of England.

II. General Observations on the Galls of layered Apple Stocks.

Our observations on crown-gall have been chiefly directed to the galls occurring on layered apple stocks, either grafted or not grafted, at the end of the second and of the third season after their removal from the parent stools. The method of vegetative propagation called "layering" involves the cutting down of the stock and the earthing up of the resulting shoots to induce them to send out roots; these rooted shoots are severed from the parent "stool" and planted out for nursery purposes (budding, grafting, etc.). It follows therefore that often some six to nine inches of stem (the portion bearing adventitious roots) are planted below ground level.

As a rule the galls found on these stocks grow out from wounds. The wound at the lower end of the shoot, caused when it is removed from the stool, is the most obvious place where internal tissues become exposed, and it is there where many of the galls arise. Such galls, referred to here as "basal galls" (Pl. VIII, figs. 1 and 2), are sometimes almost spherical in general outline, but if the stock has been planted up with a long "heel" an elongated very irregular gall may develop (Pl. VIII, fig. 3). Lateral galls growing out from the side of the stem, from the ground level to the base, here referred to as "lateral galls," are also of frequent occurrence. The basal and lateral stem galls may reach a diameter of several inches in two or three years; their surface is always irregular with numerous rounded swellings from 1 mm. (sometimes less) to 2 or 3 mm. in diameter. Smaller galls often arise on the roots, and on some of the finer roots these have the appearance of rounded nodules which may be isolated (sometimes on the broken or cut end of a root), or several may form a series along the root.

It soon became apparent that the basal galls were larger and more numerous on certain varieties of layered stocks which root from the layer with difficulty and close to the base, and which are consequently

often torn from the stool instead of being cut, as are the better rooted layers. Type VII is one of the best examples of this class. Examination indicated that the rough wound made by tearing the stocks from the stools was more liable to infection than a clean cut, and this was borne out by subsequent experimental work (14).

On each of these apple stocks raised from stools, then, there is always at least one wound, and there must often be others which occur during the subsequent handling and planting up. When later these stocks are grafted there is, of course, another wound, but this is usually at some little distance above ground, in the method of grafting adopted in nurseries in this country, and it appears to be exceptional for galls to develop round the junction of stock and scion, as only in a few cases have nodular swellings been found at the grafts.

The relation of this type of basal infection on our layered stocks to the type of infection so commonly reported in America where "bench grafting" is practised, will be obvious. In bench grafting either the whole stock or a piece of the root is grafted during the winter and "heeled in" in a shed or cellar to "callus" until the open ground is in condition to allow of planting. It is then planted rather deeply so that the union between stock and scion is completely covered with soil. It thus results that cut surfaces, not usually fully protected from infection unless special precautions are taken, are covered with sand or soil almost continuously from the time the cuts are made. If the crown-gall organism is present in either the soil or sand, conditions are favourable for its entrance into the young tree. The position of these cut surfaces in the bench grafted trees, just below ground level, gives rise to the frequent occurrence in America of galls at the "*crown*" as opposed to the basal galls of layered stocks in this country.

That the galls may increase considerably in size in the course of a year was shown by the following observations.

During the winter of 1919–20 nine young grafted apple trees were selected, some showing well-developed galls, others irregular callus development at the base suggesting incipient tumours. The parts bearing galls were photographed (natural size where possible) and a record was made of the dimensions of the galls. The trees were then planted up in a small plot at the East Malling Research Station. In the following winter (1920–21) the trees were lifted and the dimensions of the galls again taken; it was then found for example, that a lateral gall had increased from 3·5 (longest diameter) to 5·3 cm., a basal gall from 5·4 to 7·6 cm., and another basal gall from 4·4 to 6·4 cm. (Pl. VIII, figs. 1

and 2). Two of the trees had no distinct galls when planted up, but the callus at the cut end (at base) had wart-like swellings suggesting incipient galls; when examined the following season, however, these were apparently unchanged, so that the warty protuberances were probably merely irregularities in the normal callus growth.

After examination the nine trees were re-planted and again lifted in the winter of 1921–22, when the galls were compared with previous records. It was found, however, that on this occasion no further increase whatever in the size of the galls could be detected, except in the case of a small gall on a root. The trees themselves had made but little growth during the previous summer[1] and it was probably this that had had an adverse effect on the development of the galls.

When examined in the winter 1922–23, there was no further increase though the galls still showed no signs of decay, and the experiment was discontinued.

The galls found on the two, three and even four-year-old apple trees examined at East Malling, showed no signs of disintegration, but on a young apple tree received from another nursery in Kent there was a gall which was brown in parts and evidently in an advanced stage of decay. It is generally supposed that as the galls become disintegrated they liberate the gall-producing organisms into the soil which thus becomes further infested. Robinson and Walkden (7) find that in the case of chrysanthemum galls "there are enormous numbers of bacteria situated on the exterior of the gall, that a high percentage of these is *B. tumefaciens*, and that it is reasonable to assume that the presence of these progressively increasing numbers of *B. tumefaciens* on the exterior provide the progressive stimulus which leads to the continued growth of the gall." If it should prove that a similar condition obtains in such underground galls as those under consideration here, it would seem that the soil would become automatically infested in the neighbourhood of the galls while they are still intact and developing.

The consistency of these apple galls and their continued growth from year to year indicate that they are of the type known as "hard galls" by American investigators. "Soft galls" have not been seen on the layered apple stocks at East Malling. On one occasion, however, a two-year-old seedling with a well-developed hard gall was planted up in a pot, and when examined the following year, it was found that the hard gall had increased in size and was quite sound while near it there had

[1] This result was doubtless due to (1) the abnormally dry summer of 1921, and (2) the fact that trees of that age may be greatly checked by frequent transplanting.

developed a soft gall (about an inch in diameter) which was brown in colour and swarming with eelworms; whether the eelworms or bacteria were the primary cause of the soft gall was not determined.

Only one instance of what appears to be a true case of "hairy-root" (a condition commonly associated with crown-gall in America) has come under our notice on the East Malling stocks. A young apple stock, with several clusters of fibrous roots, bore at one node a tuft of roots which were finer and softer to the touch than the rest. An organism has been isolated from the swollen base from which these roots arose and is being compared with others isolated from the usual type of apple gall. The very plentiful development of fibrous roots on some of our apple stocks appears to be typical of certain varieties and, so far as we can see, has no connection with crown-gall.

III. Relative Susceptibility of the Different Stocks.

Observations extended over four years tend to show that the different varieties of layered stocks differ widely in their susceptibility to crown-gall. Some varieties show year after year not only a higher proportion of galled stocks or trees but also larger galls than others. Although the order of stocks in regard to the amount of infection observed varies in detail from one season to another, two varieties, Type VII and a layered "free" stock known to us as "No. 51," stand out as being always the most highly susceptible of all.

Those stocks of which a considerable quantity (mostly in the form of one year and two year old trees) have been available for examination may be roughly divided into three groups. In the first group, which may be called "very susceptible," come the two varieties mentioned above, Type VII and No. 51. Under the conditions in which they were grown 88 per cent. of one year old and two year old trees on these stocks showed at least a trace of crown-gall, nearly 50 per cent. of the trees could be classed as conspicuously galled, and of these 25 per cent. actually had galls one inch or more in diameter. The second group may be called "moderately susceptible"; of these the most susceptible is undoubtedly Type II (Doucin), which at times has shown nearly 80 per cent. of trees with at least a trace of crown-gall, but here the proportion of conspicuous galls is very much lower and rarely exceeds 15 per cent. of the whole, even in the case of Doucin, whilst others of the group show still less infection. The third group, which may be called "moderately resistant," includes several varieties which rarely (under our conditions) show as many as 50 per cent. of trees with traces of galls, whilst galls

one inch or more in diameter are often entirely absent and seldom exceed 2 or 3 per cent.; in this group come Type I (the "Broadleaf" Paradise), and Type VI ("Nonsuch" Paradise).

It has been already mentioned that those varieties of apple stocks which root with difficulty when propagated vegetatively show a relatively high degree of susceptibility, and this is emphasized by the grouping. Both of those in the first group are rooted only with difficulty; the same is true of the Doucin, Type II, the most susceptible of the second group. The most resistant of the third group, Types I and VI (Broadleaf and Nonsuch), are amongst the most easily rooted of all apple stocks. But this difference in rooting, and the necessity for tearing the shy rooting stocks from the stools, will not account for all the differences in susceptibility. Type VII, for instance, is much more easily rooted than No. 51, and somewhat more easily than Type II, yet it has invariably been the most susceptible. It may safely be said that there are real, inherent differences in susceptibility amongst the varieties, besides those apparent differences which arise as a result of the particular mode of removal from the stools.

A point of some interest is that there is no close correlation, so far as our observations go, between the relative "vigour" of a variety and its degree of susceptibility. Each of the three groups includes varieties widely different in their vigour; of the two most vigorous stocks, one, No. 51, is "very susceptible," whilst the other, Type XII, is "moderately resistant"; the "moderately susceptible" group includes several of the very vigorous Continental varieties of Paradise stocks, together with Type IX (the Jaune de Metz), one of the weakest growing apple stocks known. It would seem then that there are factors other than vigour that determine the degree of susceptibility of any particular variety.

This conclusion does not imply that there is no correlation between the size of the galls and the vigour of the individual trees bearing them, *within the variety*; in fact, from analogy with results obtained from experimental work on other species of plants it would appear probable that, in the case of fruit trees also, the rate at which a gall develops depends on the vigour of the host as influenced by soil conditions and other environmental factors. Levine (6) has shown that in the case of mangels infected with crown-gall the vigorous plants (grown in rich soil) produced large galls while the weak plants (grown in poor soil) gave rise to small galls.

IV. Susceptibility of the Stocks as influenced by the Scions.

In the observations described in the preceding section the possible influence of the scion with which a tree is "worked" is eliminated, since the results were all based on equal numbers of stocks grafted with the two varieties Bramley's Seedling and Worcester Pearmain. As our observations proceeded evidence was obtained that this factor, the influence that the scions may have on the number and size of the galls on the stocks, must be taken into consideration in determining the relative susceptibility of different stocks. Thus, in the case of three varieties used as scions in 1919, it was subsequently found that the galls on the stocks worked with either Lane's Prince Albert or Worcester Pearmain showed a much greater development than did those on the stocks worked with Bramley's Seedling. This point has been followed up, and large numbers of trees of many varieties have been examined each winter.

Unfortunately it has not been possible since 1919–20 to examine very many trees of any variety on a highly susceptible stock; we were thus obliged to rely for trustworthy figures on stocks which are somewhat less susceptible, such as Type II. Even here, however, the figures repeatedly show a potent influence of the scion on the development of the galls.

Of all the varieties examined, Lord Derby appears usually to render the stocks more susceptible to crown-gall than any other, followed by Early Victoria and Lane's Prince Albert. On the other hand, Bramley's Seedling, Bismarck, Newton Wonder and Stirling Castle appear to render the stocks more resistant than most other varieties.

The influence of the Lord Derby scions on stocks is correlated with a high degree of susceptibility in the variety itself, as was recently seen when some half dozen trees of this variety on their own roots (grown from layers) were lifted for transplanting. Nearly all had large galls, and none could be regarded as less than moderately galled. For so few trees they were undoubtedly one of the worst batches ever lifted at the East Malling Station. It seems likely that the Lord Derby variety (on its own roots) will prove to be even more susceptible to crown-gall than Type VII.

V. Observations on Galled Trees in the Plantation.

An exact knowledge as to the effect that crown-gall has on fruit trees in this country is wanting, and the evidence supplied by horticulturists

in America is somewhat contradictory. In some cases, however, careful records have been made by American workers on a large number of trees, some healthy and others with galls, and the results obtained go in the direction of showing that the galls have an adverse effect. One of the most recent papers on the subject is by Greene and Melhus (3), who obtained records of the rate of growth and amount of fresh growth made from year to year by young apple trees; the chief points in their summary of results are:

"In this experiment galled trees did not grow nearly as rapidly as normal trees. Those which were galled when set and later recovered made a greater growth than those that did not recover from the galls.

"The data presented show that crown-gall greatly retards the growth activities of young apple trees. It is followed by a reduction in the amount of increase in trunk diameter, and in the number of twigs, their length, their thickness and their weight."

An experiment on somewhat similar lines has been carried out at East Malling with, however, inconclusive results. Records have been made for a considerable number of trees of Bramley's Seedling and Worcester Pearmain worked on Types II and VII, some of which bore galls (the size of the gall on each galled tree was carefully noted before planting), the others being free from galls at the time the trees were planted up. In the average length of new wood formed each year, and also in the average number of fruit buds, the variation seems to be entirely independent of the presence or absence of galls at planting, and of their size and position on the stock. It was thought likely that lateral galls (on the stem, just below ground level) might have a more adverse effect than basal galls, yet the records obtained do not indicate any such difference. In one case, five trees of Bramley's Seedling on Type VII, noted at planting as having large lateral galls, actually made on the average from 25 to 30 per cent. more growth, as estimated from the length of young shoots produced, in both 1920 and 1921 than a similar group of four trees noted as being only slightly galled at the base. In other cases the difference is reversed. On the average of a large number of trees, those noted as being more or less galled when planted have made at least as much growth, and have blossomed to approximately the same extent, as those noted as "clean."

It would appear then that, under the conditions which obtain at East Malling, the galls produce at any rate no striking effect on the general health of the trees after they are planted up in their permanent quarters. The records however do not include any account of the possible

effect of the galls on the young trees previous to their being planted up. As mentioned below some 40 per cent. of the total weight of a young seedling apple tree may be gall tissue. In some Manetti rose stocks the galls have been found to be 50 per cent., or even more, of the total weight. It would appear inconceivable, in such cases, that the galls are not in some way detrimental, if only because of their absorbing food which should have been utilised in developing the normal organs, and the fact that microscopic examination shows that the cells of apple galls contain large quantities of starch is not without significance. Fracker's(1,2) observations on apple nursery stocks are of interest in this connection; in an examination of 1012 young apple trees he found that 65·1 per cent. of the non-infected trees could be classed as Grade I, while only 37·7 per cent. of trees bearing galls or infected with hairy root were sufficiently large and strong for that grade.

VI. Observations on Galls found on Seedling Apple Trees.

From our earlier observations, *i.e.* on layered stocks, there was no good evidence that the galls originated anywhere except at the wounds produced during the process of removing the stocks from the stools and planting them up, and (rarely) at the grafts. More recently however it was found that well-developed galls may occur on seedling trees in the absence of obvious wounds. Thus a crab stock was found to have numerous galls on its roots, the largest of these galls being over an inch in diameter, and there was nothing to show that the roots had been injured previous to the development of the galls (Pl. IX, fig. 4). Again, a number of two-year-old seedlings raised at East Malling from pips of cider apples have been found with comparatively very large galls (Pl. IX, figs. 5 and 6), although the trees had not been moved, since the seeds were sown, until they were taken up at the end of the second year; the galls were mostly at the "crown" of the trees, which appears therefore to be a particularly susceptible point in the case of apple seedlings. Compared with the size of the trees some of these galls were of considerable size; thus in one case a young tree with one gall was found to weigh 17·9 gm.; the gall was then cut off and its weight ascertained to be 7·8 gm. or 43·6 per cent. of the total weight of the tree. Of some 2000 seedling trees examined, barely 100 (or about 5 per cent.) were entirely free from galls —a very small proportion considering the conditions under which the trees were raised.

How infection arose in the case of such seedlings is at present not clear. Possibly the galls arose in some instances from wounds produced

during cultivation; injuries caused by wireworms and other insect larvae are also suggested as providing an entrance for the gall organism. E. F. Smith (10) has shown that in certain experiments, where artificial inoculations were made in the region of rapidly growing tissues, "secondary galls" arise at some distance from the wound where the "primary gall" develops. Although his interpretation of their mode of origin is criticised by Robinson and Walkden (7) they confirm his observations on the fact that the secondary galls arise from within and in the absence of any external injury at those particular points. Whether galls arise in a similar manner in the more woody tissues of apple roots is not known, but the way in which the galls sometimes arise in series along the roots on seedling trees, and occasionally also on layered stocks, suggests that perhaps some of them arise as secondary galls.

VII. Aim and Scope of Work in Progress.

The field observations described in the preceding pages have suggested lines for further investigation, and research is in progress to obtain information on the following points.

(1) *The Cause of the Galls.* That the galls occurring on apple stocks in Britain are a result of the invasion of the tissues by a micro-organism is suggested by their close resemblance to the galls found on nursery stocks in America where investigation has shown them to be due to the presence of *Bacterium tumefaciens.* Bacteria, which, in preliminary cultural tests[1], show affinity with *B. tumefaciens*, have been isolated from apple galls found on layered apple stocks at East Malling, and inoculation experiments carried out with pure cultures on young apple stocks have yielded, in certain cases, tumourous outgrowths; these positive results, however, up to the present, are too few in number for definite conclusions to be drawn as to the parasitic nature of the organisms isolated, and further inoculation experiments are being carried out. That *B. tumefaciens* does occur in this country was announced in 1921 by Walkden (13), who isolated it from galls found on *Chrysanthemum frutescens.*

As already mentioned, tumours of the crown-gall type have been found on a number of species of cultivated plants in this country. Whether all such galls are caused by micro-organisms and whether the parasite, if present, is, in each case, identical with the American species, *B. tumefaciens*, are points which require elucidation.

[1] The preliminary cultural and inoculation experiments were carried out at the South-Eastern Agricultural College, Wye, and the work is being continued at the East Malling Research Station.

(2) *Effect of Galls on the Vigour of Nursery Stock.* It has been shown that no essential difference has up to the present been detected at East Malling in the general vigour of trees subsequent to their being planted up with or without galls. Further work is required, however, particularly as to the effect the galls may have on the young ungrafted trees, and also on the grafted trees while they are still in the nursery. Reference has been made to Fracker's observations, but the results need to be confirmed by direct experiment using uniform stocks. Material for such an experiment is available at East Malling, and when a method of inducing gall development as required has been found, a comparison will be made of infected and uninfected young stocks.

(3) *Control Measures.* The fact that, in the case of layered apple stocks, the galls arise chiefly at wounds suggested that a possible method of control lay in reducing to a minimum the surfaces liable to infection. Field experiments with this object in view were initiated in 1920, and from the results of experiments already carried out(14) it would appear that the number and the size of the galls may be reduced by avoiding unnecessary injuries, and by covering the wounds (made when removing the stocks from the parent stools) with a protective layer of Stockholm tar or grafting wax. A more detailed account will be published when the results of a further series of experiments, now in progress, are to hand.

Again, since infection occurs on planting the stocks in infected soil, it appears probable that the crown-gall organism is capable of living saprophytically in the soil, so that another possible means of control is in a suitable treatment of the soil, either by judicious manuring, or by the application of a bactericidal substance. Whether treatment along these lines will prove practicable remains to be proved, but biological studies of the apple crown-gall organism may indicate the best way of approaching the problem.

(4) *Identification of Incipient Galls.* Another feature of interest to the plant pathologist is the diagnosis of the disease in its early stages. The irregular callus that develops over a ragged wound may resemble an incipient gall, and such irregular callus development is frequent on nursery stocks which are propagated vegetatively, especially if the shoots are torn away from the stools. At what stage it is possible to distinguish between a young gall and normal callus has not yet been determined, but when a successful method of inducing galls on apple stocks by artificial inoculations has been found a comparison of young galls and the uninfected callus of control plants will be made.

SUMMARY.

An account is given of the galls ("crown-gall") found on layered apple stocks and on seedling apple trees raised at the East Malling Fruit Research Station.

On the layered stocks the galls arise most frequently at the base of the stocks, *i.e.* at the wound caused when removing the stock from the parent stool, but they may also occur on other parts of the stem below ground, and on the roots.

Observations extending over several years lead to the conclusion that, although galls have been found on every variety of layered apple stocks so far propagated at East Malling, some varieties are far more susceptible to crown-gall than others.

Evidence has been obtained that the susceptibility of the stock is to some extent influenced by the variety of scion grafted on it.

No difference in vigour could be detected on the subsequent general growth of trees planted up with, or without, galls.

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Fig. 2.



Fig. 1.

WORMALD & GRUBB.—The Crown-Gall Disease of Nursery Stocks. (pp. 278—291.)



Fig. 3.



Fig. 4.

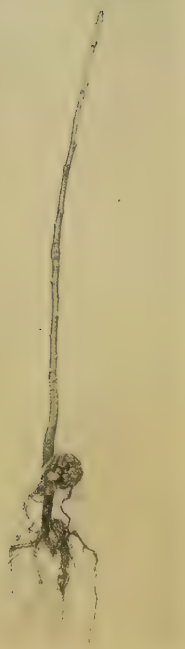


Fig. 5.



Fig. 6.

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EXPLANATION OF PLATES VIII AND IX.

PLATE VIII.

Fig. 1. A typical basal gall on a layered apple stock. (Nat. size.)

Fig. 2. The same a year later. (Nat. size.)

PLATE IX.

Fig. 3. A stock torn with a long "heel" from the stool; galls are developing all along the wound. $\times \frac{3}{8}$.

Fig. 4. The root-system of a crab stock badly galled. $\times \frac{1}{4}$.

Fig. 5. A two-year-old apple seedling tree with a gall at the collar, showing relative size of tree and gall.

Fig. 6. The gall (seen in Fig. 5). $\times \frac{5}{7}$.

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SOME OBSERVATIONS ON THE GROWTH OF *RHIZOCTONIA CROCORUM* (PERS.) DC. IN PURE CULTURE

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(With Plate X and 3 Text-figures.)

Name of the fungus.

The fungus with which these notes are concerned is that which causes the well-known "Violet Root Rot" of various crops, such as lucerne, potatoes, carrots, etc., and which has hitherto been generally known in this country as *Rhizoctonia violacea* Tul. The specific name *violacea* was given by the Tulasne brothers (1) in 1851 when they reduced to a single species the older forms known as *R. Crocorum* and *R. Medicaginis*. This proceeding is however contrary to the Rules of Nomenclature now accepted. Assuming, as do the majority of workers, that the Tulasne brothers were right in their view that the violet root disease in all the various crops is caused by one and the same species of fungus, even though there may be biologic forms, then the earliest valid name, and not that of the Tulasnes, must be adopted. As pointed out by Duggar (2) the correct combination is *Rhizoctonia Crocorum* (Pers.) DC. The specific name was originally used as *Sclerotium Crocorum* by Persoon (Synopsis, 1801) for the form on *Crocus sativus*. It was transferred to his genus *Rhizoctonia* by De Candolle in 1815, and both this and De Candolle's second species, *R. Medicaginis*, were taken up by Fries in the Systema Mycologicum (1823), which latter is the starting point for Nomenclature according to the International Rules. It is unfortunate that the name is less descriptive than *R. violacea*, and is particularly inappropriate in this country where the Crocus disease is unknown. Pending further information as to the perfect stage, however, the name *R. Crocorum* must stand.

Origin of the present work.

In the winter of 1922–23 a considerable amount of circumstantial evidence was obtained pointing to the possibility that *R. Crocorum* might

have as its perfect stage the fungus *Helicobasidium purpureum*. The authors therefore set out on an attempt to test the theory by pure culture and inoculation experiments. They were fortunate in being able to obtain comparatively abundant material of the *Rhizoctonia* on clover and later also of *Helicobasidium* through the kindness of Mr W. M. Ware of the South-Eastern Agricultural College, Wye. Owing to unexpected complications it has not yet been possible to obtain sufficient evidence for a definite pronouncement, but the authors hope to be able to give some account of this investigation after a more complete set of inoculation experiments has been carried out.

The present notes are the outcome of one side of the work, namely, the successful and continued growth of *R. Crocorum* in pure culture. The only previous records of artificial cultures of this fungus known to the authors are those of Diehl(3) in America and van der Lek(4) in Holland, neither of whom carried out very extensive cultural work with it. The former grew the *Rhizoctonia* chiefly in mixed culture with a species of *Fusarium* and did not obtain good growth in the pure state. Van der Lek succeeded in isolating the fungus pure from sections of the "infection cushions" or "corps miliaires" and obtained good growth on malt agar. He also grew it on cherry and dung extract agars and gives some account of variations in colour according to the medium and the amount of moisture present. We understand that both of these workers allowed their cultures to die out. Faris(5) writing in 1921 stated that "cultural experiments to secure pure cultures of the organism (*R. Crocorum*) are under way" but the authors have seen no account of successful cultures. In a letter to one of the authors Dr G. H. Pethybridge stated that he grew the fungus successfully, although not in pure culture, by keeping an attacked potato tuber in a covered dish with other healthy potatoes. In time the *R. Crocorum* grew luxuriantly over the whole lot and its "corps miliaires" penetrated the skins.

Methods used for isolation in pure culture.

After numerous unsuccessful attempts in the winter of 1922–23 growth of *R. Crocorum* was obtained in pure culture on malt agar and on a meat and malt extract gelatine acidified with citric acid[1] by adopting the method of van der Lek(4), viz. planting moderately thick sections of "infection cushions" of the fungus, cut with a razor under aseptic conditions, on previously poured plates of the media. One

[1] This gelatine was made up with tap water 1000 c.c., malt extract 20 gm., Lemco meat extract 10 gm., citric acid 4 gm., gelatine 120 gm., and cleared with white of egg.

of the sections showing promising growth was sacrificed for high power microscopic examination and the developing hyphae were seen to be growing out from the centre of the micro-sclerotium as described by van der Lek. The material used was that on Red Clover, mentioned above, supplied by Mr W. M. Ware (cf. (6))[1]. Van der Lek (*loc. cit.*) gives no details of his method of surface sterilisation of his plant material. The authors obtained best results from the use of a 50 per cent. alcoholic 1/1000 mercuric chloride solution with technique similar to that adopted by bacteriologists (cf. Manns (7)). Portions of the clover root bearing numerous infection cushions or micro-sclerotia were put into sterile test tubes and well covered with the mercuric chloride solution. After a given interval the disinfectant was poured off and the material washed at least three times with separate portions of sterile distilled water. Each time the liquid was shaken to all parts of the tube and on changing the liquid the old cotton wool plug was replaced by the dry sterile plug from the new sterile water tube. The material remained in the original tube throughout the operation. It was found that about one minute in the mercuric chloride solution was ample to kill all really surface contaminations: longer exposure usually resulted in absolutely sterile sections—killing also the micro-sclerotia of the *Rhizoctonia*. After surface sterilisation the material was transferred to a sterile petri dish, portions cut off from the root tangentially $\frac{1}{2}$–1 mm. thick and transverse sections of the micro-sclerotia cut in sterile pith. The razor was flamed after dipping in alcohol before use. The difficulty which has been experienced by previous workers (cf. Duggar, *loc. cit.*) in obtaining growth of *R. Crocorum* on artificial media has been occasioned in part by the apparently low degree of vitality possessed by such minute portions of mycelium as can be transferred without accompanying contaminations from the advancing edge of growth of the fungus, *e.g.* at the collar of infected plants. In the opinion of the authors the main difficulty has been due *not* to the fungus being on the borders of obligate parasitism but to the difficulty of seeing and isolating the hyphae, which grow out slowly from viable portions of the fungus, *e.g.* the micro-sclerotia, before they have been swamped by other more quickly developing fungi and bacteria. In the field it is rare to find many plants attacked by the parasite and the choice of material is rather limited. Further, from the fact of the *Rhizoctonia* occurring mainly on the underground parts of plants, it is

[1] A further case in this country of a very slight attack of *R. Crocorum* on Red Clover has since been noted by us in Hampshire. *R. Crocorum* has also occurred in one of our experimental pots on another species of clover (*Trifolium hybridum*).

always associated with very numerous rapidly growing saprophytes and in many cases before the attacked parts are dug up these saprophytes have already obtained a hold on the tissues affected by the *Rhizoctonia*. In cases where the razor sections of the micro-sclerotia were "planted" free from contaminations and where the surface sterilisation of the roots had not been too severe the *Rhizoctonia* grew out slowly but steadily on to the artificial medium[1]. When suitable conditions were found for the growth of the fungus it grew at least as freely in culture as some other parasitic fungi whose occurrence on the aerial parts of plants comparatively free from contaminations, combined with the production of abundant conidia, has led to their being isolated in pure culture without difficulty. The very slow growth of certain parasites, such as species of *Pseudopeziza*, in artificial culture has been noted particularly by Klebahn (8).

Confirmation of the identity of the organism isolated.

The mycelium which grows out from the infection cushion or micro-sclerotium is at first colourless, and the hyphae very slender, but very soon the characteristic features of the branching of *Rhizoctonia* can be detected. After the lapse of two to three weeks at laboratory temperature the colony shows the typical violet colour and the older hyphae are now seen to be assuming the size and colour characteristic for *R. Crocorum*. The centre of the colony gradually becomes deep violet or brownish, according to the medium, and at the same time the substratum becomes permeated with the densely matted branching hyphae, so that a tough, compact mass is formed which is very difficult to cut with the usual platinum needles used for making sub-cultures.

The hyphae of *R. Crocorum* obtained in culture do not differ essentially from those of the fungus in nature, as described and figured by Duggar (*loc. cit.* p. 415). The normal well-grown hyphae are usually about 4–6 μ in diameter. The younger hyphae are pale, especially towards the tips, with rather distant transverse septa. At the apices the hyphae contain dense protoplasm, but further back they become vacuolate, while older hyphae become thick-walled, rigid and deeply coloured, and appear to contain little or no protoplasm. Branches arise usually singly, more or less at right angles to the main hypha, and near to the

[1] A contributing factor to the slow growth obtained at first was that it was necessary to use a fairly thin layer of medium, with consequent drying out, in order to be able to watch the growth with the low power of the microscope through the bottom of the petri dish.

cross walls. Each branch is cut off by a septum close to the main hypha, and frequently appears to be slightly narrower near the point of origin. Duggar's observation that at the point of union the diameter of the branch is uniform with that of the main hypha applies more to the older, coloured hyphae. The experience of the writers has been that the younger hyphae, both in nature and in culture, usually show this slight narrowing where the branches join the parent hyphae. The same observation has been made by Faris(5). Occasional fusions between adjoining hyphae take place, but no clamp connections have ever been observed.

In order to confirm the results of microscopic examination and to show more definitely that the fungus isolated was really *R. Crocorum* inoculation experiments were carried out. As a preliminary test, on 29th July, 1923, portions of mycelium from a pure culture were placed in contact with young carrots and potatoes, the surface of which had been carefully washed, treated with dilute formalin and again washed. Two potatoes and three young carrots thus inoculated were kept moist in a large covered glass dish. In the case of the potatoes there was a very slow growth of scattered hyphae over the surface of the tubers but no further development. With the carrots, however, better results were obtained. On 1st September one of the carrots showed a distinct patch about 1 cm. in diameter of typical *R. Crocorum* attack with minute infection cushions, while a second root showed also incipient attack and a few infection cushions. In both cases the affected parts became gradually much larger until finally both carrots were completely destroyed. Secondary organisms no doubt contributed to the rot.

A further inoculation experiment was carried out with young growing carrots. Some seedling carrots had been carefully transplanted into two large pots of soil which had been sterilised by steam by the usual method for gardening operations. By 30th July these were well established and sufficiently large for inoculation. In one pot fairly big pieces of growth cut from pure culture were inserted in the soil close to the crown of each plant. The second pot was left untouched as a control. By 17th September one carrot in the inoculated pot was seen to be dying off and when pulled up was found to be covered with the typical infection cushions of *R. Crocorum*. By 18th October a second carrot had completely rotted and the skin remaining was found to be full of infection cushions. Later a third carrot died and was found to be attacked in the same way, while another showed slight infection. In the control pot no sign of *Rhizoctonia* ever occurred. From one of the infected carrots the parasite was re-isolated by the same method as used originally.. The

growth in artificial culture was found to be exactly similar to that obtained from the clover material.

A similar inoculation test was carried out with Red Clover. Seeds of Red Clover had been sown in large pots in sterilised soil in July 1923 and on 3rd September the young plants in one pot were inoculated by placing portions of a pure culture in the soil close up against the upper part of the tap-root of each plant. On 2nd November further mycelium from a pure culture was introduced. A pot was left uninoculated as a control. The pots were kept in a small unheated glasshouse, and as from the time of the first inoculation the weather was never warm results were doubtless obtained much more slowly than they would have been had it been possible to inoculate earlier in the season. On 11th April 1924 all the clover plants were pulled up and examined. Of those in the inoculated pot five out of eight showed typical attack by "Violet Root Rot." Infection cushions occurred both on the tap-roots and on some lateral roots, while in one case the tap-root had completely rotted away leaving only a short stump. The clovers in the control pot were found to be all quite healthy.

There seems to be no doubt therefore that the organism isolated and grown in pure culture is really *R. Crocorum*.

The successful inoculation of carrots with a pure culture obtained from clover plants is of some significance in connection with the view expressed by Eriksson (9) that the violet root rot of the carrot and of lucerne (a legume) are two entirely different things. Eriksson considered that the sterile fungus called *R. violacea* (*R. Crocorum*) might include several biologic forms or even species. He based this opinion in the first place on the results of some cultural experiments. Using infected soil in which a crop of diseased carrots had been grown, he got only a slight infection on lucerne and none on Red Clover or parsnip; in one experiment made with cross-inoculation in the reverse direction, from lucerne to carrot, he got no result. Similarly a test with forms of the fungus on lucerne and asparagus indicated that these would not pass from one host to the other. From these experiments and from records of the occurrence of the alfalfa (lucerne) disease and of attacks on Red Clover in Germany (the former frequent, the latter rare) he argued even that the lucerne and the clover forms might be distinct.

Seeing that our present knowledge is so slight, however, both as to the requirements of the fungus and the conditions necessary for successful infection of its various hosts, Eriksson's experiments require to be carefully repeated.

Eriksson's further argument that the perfect stage of the carrot fungus is his *Hypochnus violaceus* and that of the alfalfa fungus *Leptosphaeria circinans* is generally regarded as being based on insufficient evidence, and need not be considered here.

Growth on various media.

Diehl (3) obtained his best growth of *R. Crocorum* on "radicicola" agar acidified to 5 on the scale given in old editions of the American Standard Methods of Water Analysis. As the authors were unable to have access to an old edition it was impossible to find how much added acetic acid was represented by the number 5, but from the description given by Diehl it would appear that very much better growth was obtained on the media generally used in the present work than that given by the acidified "radicicola" agar. Van der Lek obtained good growth on malt agar.

The authors have found that media made up with malt or meat extract or preferably both, and also prune agar, allow of best growth, and these have been chiefly used. When first isolated, however, transfers were made to a great variety of media with the aim of finding as early as possible which would be the most suitable, so that there should be no risk of the organism dying out when once obtained in pure culture. Pure growth was sparse at the time so that only a few tubes of each were inoculated. Further, in some cases the agars available had been prepared for some time, and it has been noted from later work that there may be appreciable differences, particularly in the amount of aerial growth, when freshly tubed agars are used. The results are, however, given here in brief in order to show on how wide a range of sterilised artificial material growth of *R. Crocorum* will take place when it is not overwhelmed by the presence of other more rapidly growing organisms.

As an illustration of the way in which the development of aerial mycelium, and hence the general appearance of the culture, depends on the amount of condensation moisture present one case may be given where four malt-meat extract agar plates were poured at the same time, and inoculated with as nearly as possible equal and very minute fragments of mycelium from the edge of a recent culture. The plates were kept in an incubator at 22–23° C. It happened that two of these plates included a considerable amount of water of condensation while the other two remained fairly clear and dry. After about four weeks the differences in growth were striking. In the two plates with much moisture

there was good development and a soft growth of pale lilac-brown aerial hyphae similar to that occurring in fresh tubes. The plate with most free moisture gave a colony 25 mm. in diameter while the one with slightly less moisture measured 13 mm. The other two plates, which had remained clear and dry, showed very much less growth less than 10 mm. in diameter—and the mycelium was practically entirely submerged, the colony therefore appearing dark purple in colour with no paler aerial growth. On the other hand, while endeavouring to obtain conditions under which the fungus would give regular and equal growth on duplicate plates in artificial culture it was grown on malt extract and meat-malt extract agars made up with 1, 1½, 2, 3 and 5 per cent. of agar, and the varying percentage of contained agar *per se* did not appear to have any appreciable effect on the growth of the organism either at 19° C. or at 30° C.

The microscopic characters of the hyphae may vary somewhat according to the amount of moisture available. In vigorous cultures on freshly-made agars the marginal hyphae grow out fairly straight, with rather infrequent branches. On plates which are drying out, on the other hand, the hyphae become more flexuous and branching is more frequent. Finally in the compact tissue of cultures some weeks old irregular swollen cells may be found resembling those figured by Duggar (*loc. cit.* p. 418, fig. 3) as covering the surface of large sclerotia.

Bodies resembling sclerotia in form occasionally occur, especially as cultures dry out, but the structure of these is more compact and consists of more regular hyphae than Duggar describes for the large sclerotia occurring in nature. The surface of such bodies is, however, often covered like the sclerotia with a layer of short, irregularly branched and thickened hyphae. These sclerotia-like bodies found in cultures appear to be extreme developments of the compact, heaped up growth which occurs at low temperatures rather than special structures such as the sclerotia of *Botrytis*, etc.

Duggar notes that the violaceous pigment of the young hyphae as found in the field is soluble in acidulated water. The authors found that when the growth was reasonably well aerated, as a general rule, abundant pigment was formed and this readily diffused out into the acid gels or liquid cultures. In the latter where the submerged mycelium could be observed it usually appeared colourless, however deeply coloured the medium or the surface growth might become. In this connection it is interesting to note the statement of Faris that "very little pigment is produced after the mycelium enters the host." The absence of colour

within the host tissues has also been noted by previous workers. The colour in liquid cultures was found to be unaffected by heat and to be insoluble in such solvents as ether, chloroform, etc.

With many of the liquid and some of the agar cultures rolled filter paper, and pieces of stick or glass rod, were placed in the flasks before sterilisation with a view to giving the organism an opportunity of climbing up out of the medium to produce fructifications in the free air. Mycelium grew freely upwards on these supports but in no case has any sign of fructification been observed either on such growth or in ordinary cultures.

Malt agar. Growth fairly good, more brownish than purple. The mycelium piles up into compact growth several millimetres above the original level of the agar and in fresh tube cultures some loose aerial mycelium is produced. In petri dishes, where less moisture is present, the superficial growth is more even, compact and granular in appearance. In old colonies the surface growth becomes light brown, sometimes almost white. Deep brownish colour is developed in the medium, but not extending far beyond the edge of the colony. In a flask culture, after $8\frac{1}{2}$ months at laboratory temperature the agar has turned a deep brown colour and the mycelial growth has become piled up into warted excrescences nearly 2 cm. in diameter. The surface of these excrescences changed in appearance from time to time, sometimes appearing deep brown, almost black, in colour and occasionally extruding drops of brownish liquid; at other times it became lighter in colour with a more pruinose appearance, and occasionally a violaceous tint. Extending irregularly over part of the surface is loose rusty brown coloured mycelium. Similar brown mycelium extends up the glass walls in places to a height of 2–3 cm.

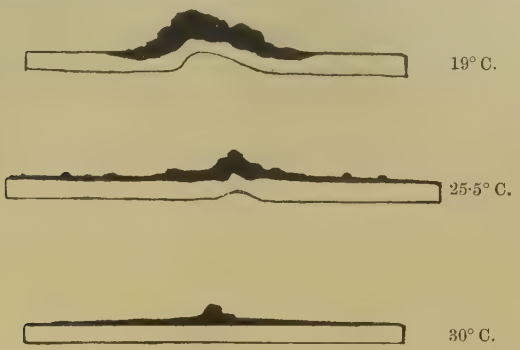
Malt and meat extract agar. Growth good—definitely the best observed. With freshly sterilised moist agar a fair amount of loose aerial mycelium develops. The colour is variable from browns to the typical violet colour of the organism. A deep purplish or brownish colour is developed in the medium. On fairly thick layers of agar in petri dishes growth appears fairly regular and dense over the whole colony at 30° C. but the violet tinge to the brown colour is shown best at 25° C. where the growth aggregates into small rounded sclerotia-like masses 2–3 mm. in diameter. At 19° C. the growth tends to pile up at the middle of the colony with the excretion of numerous drops of dark brown liquid and is very noticeably less dense than at higher temperatures over the wide outer zone of the colony. Plate X shows the appearance of typical colonies grown at these temperatures for five weeks. "Staling" has begun—particularly at 25·5° C.—as indicated by the irregular edge of the colony. In Fig. 1 vertical sections through the middle of each of these plates are shown, in order roughly to compare the total amounts of growth. This however is only approximate, as owing to the very dark colour produced in the medium it is impossible to fix exactly the limits of the submerged mycelium.

The growth of *R. Crocorum* on malt-meat extract agar does not appear to "stale" the medium for the growth of *Penicillium* sp. as infections will grow actually on the old colony of the *Rhizoctonia.*

Malt and meat extract solution. Growth very good, commencing at once from the inoculum. It was noted that growth of a single colony per flask was no more rapid

than occurred from each colony when the inoculum broke up into many pieces and many colonies were formed. The mycelium appears colourless through the bottoms of the flasks although deep colour is developed in the medium. The first aerial hyphae to appear above the surface of the solution are colourless but after an "island" of growth has been built up the surface mycelium varies in colour from brown to purple. On filter paper supports growth is poor, but on wood it spreads until the whole block is covered with a purple-brown felt—compact above but showing considerable loose mycelial development near the surface of the liquid. Eventually small bodies resembling sclerotia (see p. 299) appear near the upper end of the wood.

Malt and meat extract gelatine acidified with citric acid. Growth good with development of an intense purple colour in the medium. At the top delicately violaceous close aerial mycelium is produced. The gelatine is slowly liquefied. In some tube cultures started during a spell of hot weather the inoculum became soon submerged owing to



Black = visible compact fungus tissue. White = agar, more or less coloured.

Fig. 1. Drawings to show the comparative amounts of growth over the surface of the colonies of *R. Crocorum* grown for five weeks at the temperatures indicated. The lengths of sections indicate the comparative diameters of the colonies. The black indicates the amount of visible very compact fungus tissue. At 19° C. the growth over the outer zone of the colony was too thin to be indicated exactly proportionally.

the melting of the gelatine and in these cases no colour developed until the mycelium grew up to the aerated zone. In a flask culture started on 23rd May, 1923, and kept in a rather cold laboratory the growth was as above at first—good, with the development of much colour and some purple aerial hyphae and slow liquefaction of the gelatine. After a week or two the whole of the gelatine melted during some unusually warm weather and then remained continuously liquid with the growth submerged. All the liquid steadily developed a very intense brownish purple colour. After some eight months from inoculation an "island" of growth 3–4 cm. in diameter appeared which was opaque white, but which gradually turned to a violaceous colour. Seen through the bottom of the flask the submerged mycelium appeared colourless.

Meat extract agar. Growth similar to, but not so abundant as, that on malt or on malt and meat extract agars. On thin layers of agar in plates which begin to dry out before growth has advanced very far a tendency to form the compact sclerotia-like

bodies mentioned above has been observed. The colour developed in the agar is more distinctly purple usually than when malt is present.

Jardox extract gelatine. Flask culture. Growth very slow, almost entirely submerged with only slight development of aerial mycelium. Gelatine is very slowly liquefied. After eight months at low laboratory temperatures the liquefied gelatine has a colour somewhat similar to that of a prune decoction. The mycelium appears colourless and the pigment developed in the gelatine has not extended into the non-liquefied portion.

Jardox extract broth. Very slow growth, the liquid turning deep brown.

Bouillon gelatine (not neutralised)[1]. Incubated at 19° C. there is no liquefaction of the medium in 3–4 weeks. At 22–23° C. there is slight liquefaction in the centre of the colony. Growth is fairly good but there is comparatively little pigment production. The main part of the colony is reddish brown in the medium with a practically colourless outer zone. Some practically white aerial mycelium is developed in the middle.

Prune agar. At laboratory temperatures growth is slow and of a dingy purple colour. Old cultures show a compact somewhat granular looking surface layer with practically no fluffy aerial hyphae. On freshly made agar incubated at 22–23° C. growth is much better and equal to that on malt agar. The mycelium is dark purple in colour, and the colony is surrounded by a deep claret-coloured zone of diffused pigment.

Soft acid cherry decoction agar. Fair growth. After seven months in flask culture a deep brown colour extended about 3 cm. deep into the agar. Some close "woolly" aerial mycelium, very light brown in colour, was developed, but over two-thirds of the surface it was so scanty that the dark colour of the agar showed through.

Quaker Oat agar. Growth good. Aerial hyphae short and tending to form minute light brownish clusters which were at first taken for an impurity.

Thin oat agar. Growth very slow. Eventually there is a fair amount of pale cinnamon-coloured aerial mycelium and a deep red-brown colour is developed in the medium.

Corn-meal agar. Growth exceedingly slow and scanty, scarcely visible after four weeks at 22–23° C. A deep violet colour diffuses from the inoculum into the fresh medium.

Potato agar. Very little superficial mycelium; moderate growth in the agar developing to a very deep brown colour. In plates incubated at 22–23° C. the colony shows at an early stage an irregular fan-like development.

Sterilised potato plugs. Growth very slow. The surface of the block becomes gradually covered with a deep brownish felt, with very little erect aerial mycelium. Eventually the mycelium extends into the cotton wool on which the block rests. Very old cultures show the potato completely penetrated by the mycelium and small aggregations of deep brownish mycelium here and there over the surface.

Steamed rice. Growth slow, with dull reddish mauve colour developed in the matrix.

Bread paste. Similar to growth on steamed rice, eventually the whole matrix being penetrated by mycelium.

[1] This was adapted from the medium used by Biourge (1923) for cultivating species of *Penicillium* and was prepared according to the formula: water 1000 c.c., Lemco 10 gm., peptone 10 gm., sodium chloride 5 gm., gelatine 120 gm., pure glycerine 24 gm., cane sugar 50 gm., cleared with white of egg.

Starch agar. Growth very slow with little superficial mycelium. The typical violet colour of the organism diffuses into the agar.

Dox's agar. Very slow and almost entirely submerged with only slight aerial growth: deep colour production in the agar. After ten months a considerable number of golden yellow crystals were found on the surface of the agar.

Fermi's solution and Uschinsky's solution. Practically no growth.

Milk. The inoculum sank and although there was a fair amount of growth after nine months comparatively little colour developed. The culture appeared almost white at the base with the remainder a dirty brown colour. The top $\frac{1}{4}$ inch remained liquid but below was an almost solid plug of growth. In a litmus milk tube growth occurred, by chance, at the surface and under the aerated conditions intense colour developed similar to that with the acidified malt and meat extract gelatine. The small amount of compact aerial mycelium was light violaceous in colour with some extruded drops.

Manure extract agar. Poor growth almost entirely submerged.

Peptone agar. (An acid medium developed by Waksman[10] for counting soil fungi.) Moderate growth in the agar with production of dark brown colour. In a freshly sterilised tube there was a fair amount of light brown aerial mycelium.

Clover leaf and stem extract. Very moderate growth.

Clover leaf and stem extract agar. Growth slow, not piling up above the surface at all. There is a slight development of aerial mycelium over the whole colony, giving a delicate violaceous tint viewed from above.

Sterilised clover stems. Growth very poor and soon ceased, probably owing to rather dry conditions.

Steamed oat straw and steamed wheat straw. Very slight growth.

Crocus corms. (Not *Crocus sativus*, but the ordinary English garden Crocus.) On corms which had been sterilised in mercuric chloride solution and washed with sterile water no growth at all occurred when inoculated and placed in moist chambers. This result may have been influenced by the degree of maturity of the corms, which were specially lifted early for use, or it may have been due to the species of Crocus used. On sterilised Crocus corms growth was slow and very light coloured at first but fairly abundant after ten weeks at 30° C., forming dark brown hummocks on the surface of the corms and appearing rusty brown in colour where it extended to the sides of the tubes. The brown colour extended throughout the moist cotton wool plugs at the base. Chopped up Crocus corms used for the preparation of a Crocus decoction were also sterilised in a flask and inoculated. On this rather poor nutrient material growth was very slow and chiefly of a rusty brown colour.

Crocus agar. Moderate growth, very deep brown with little aerial mycelium.

Thick Crocus decoction. Fair growth, liquid almost black, aerial mycelium rusty brown.

The optimum temperature for growth in culture.

When first isolated growth on all media and at the temperatures tried appeared to be extremely slow. At summer laboratory temperatures sufficient growth for making sub-cultures was obtained only after several weeks. It was desirable to obtain some rough idea as to the optimum

temperature for growth of the fungus, but before our experiments yielded reliable figures the slowly growing organism also served to emphasize the importance of the "internal condition" of fungi used in such physiological determinations. Owing to the slowness of growth of the *R. Crocorum* in our original transfers and the impatience of the authors the first attempts at obtaining some idea of the temperature relations of the fungus were made by inoculating poured plates of media with pieces of growth taken from a tube culture. The toughness of the growth and the paucity of the margin of advancing mycelium make it difficult to transfer any number of approximately equal sized pieces of mycelium or without varying quantities of the medium attached. The results obtained were most irregular, especially when a portion of agar not already thoroughly permeated by the mycelium was transferred. In that event frequently some considerable time elapsed before the fungus grew out from the inoculum on to the fresh medium—in one extreme case not doing so until after the lapse of 45 days.

It may be mentioned here that in the course of the work it has been noted also that the time that elapses before new growth starts in a sub-culture depends on the age of the culture from which the inoculum is taken. If a culture about six months old is used as parent it may be two or three weeks before any new growth can be detected. On the other hand, transfers from the growing margin of a comparatively recent culture (6–8 weeks) grow out almost at once, especially if a very small piece of inoculum is used and so placed that the mycelium is immediately in contact with the fresh food material.

Edgerton(12) in his work on the temperature relations of various strains of *Glomerella*—a comparatively rapidly growing fungus—recognised the error arising from differences in the "internal condition" of the portions of mycelium used as inocula. Using "a small bit of mycelium from the edge of a rapidly growing colony" he states that in taking his measurements "the growth of the first 24 hours was never considered." In our experiments with *R. Crocorum* the error due to this cause was so considerably magnified that, as mentioned above, using transfers of pieces of mycelium from a tube culture duplicate plates varied so much as to make the results quite worthless. Examples of such irregular duplicates—each pair being the diameters of colonies in millimetres after the lapse of the same interval of time and with other conditions equal, but the inocula being different portions from the same stock culture tube—are as follows: 26 and 53; 34 and 48; 9·5 and 28; 16·5 and 29. When, however, more elaborate technique was adopted perfectly con-

sistent results were obtained. The figures given in Table I illustrate the good agreement that was obtained on measuring the diameters of colonies on series of similar plates. Plate X further shows the regular nature of the growth in the colonies although by the time these photographs were taken (five weeks after inoculation) "staling" had set in and the margins of the colonies were becoming irregular.

Table I.

Diameters of colonies in millimetres 14 *days after inoculation, on meat-malt extract agar.*

(Each figure is the average of two measurements to the nearest ½ mm. in directions at right angles.)

Incubated at 19° C.	20	19·45	17	17	18·25	18·25	18·25	17·5	Av. 18·2	
25·5° C.	24·75	22	22·5	23·75	22·75	24·25	25·25		Av. 23·6	
30° C.	18·75	21	22·25	18·75	20·75	20·75	21	20·5	Av. 20·5	

Meat-malt extract agar was used as this was found to give the best growth of any of the media tried. In order to obtain a moderately deep layer of agar 30 c.c. were used to a petri dish 4 in. in diameter—the dishes being a good flat-bottomed sample. The method of inoculation was that used by Fawcett(11), equal sized slabs 2·5 × 3 mm. in diameter being cut with a Keitt platinum cutter round the edge of a regularly growing colony in a petri dish and one of these slabs being inverted on the middle of each fresh plate of medium. The fungus grew out on to the fresh agar in all cases practically at once and it was not necessary to discard any of the plates as possibly giving erroneous measurements. Table II shows the average increases in the diameters of colonies for successive weekly periods with one set of plates incubated at the temperatures given. Each colony was measured in two directions at right angles to the nearest ½ mm. and each figure is the average for seven or eight plates at each temperature. The laboratory temperature to which the one group of plates was submitted was rather low and variable, ranging from 7° to 15·5° C. and averaging about 11° C. over the 24 hour day and night period. A set of colonies which had been grown at a temperature of 30° C. for 3–4 weeks was transferred to an incubator at 37–40° C. No growth at all took place at this temperature and the young hyphae appeared to be killed by the exposure as on transferring to 25° C. no growth took place at the edges of the colonies. After 10 days at 25° C., however, a striking growth of almost colourless hyphae was taking place from the middle zones of practically all the colonies. This greater resistance of the median zones of the colonies seems to agree

with the results of attempts at isolation—the more resistant and viable portions of the fungus being found to be the sclerotium-like bodies.

Fig. 2 shows graphically the relation between the diameters of the colonies and the temperatures at which they were grown at times three and four weeks after inoculation of the plates.

A second set of plates was started on meat-malt extract agar on a batch of medium which had been prepared at a different time from that used for the above and the inocula were from a different secondary stock culture plate. There were nine or ten plates in the group for each temperature and the results obtained were exactly similar to those given in Table II for the first set. The average figures for this second set are shown graphically in Fig. 3. It is regretted that with the limited amount of apparatus at the disposal of the authors it has not been possible to obtain the growth figures at definite 10° C. intervals so that no temperature coefficients can be calculated.

It will be seen from the results that at all temperatures, except the low variable laboratory temperature, the rate of growth increased with each seven-day period until the end of the third week from inoculation. The rate of growth then steadily decreased, the decrease being most rapid and the "staling" most marked at 25·5° C.

Table II.

Average increases in diameter in millimetres for successive seven-day periods of colonies of Rhizoctonia Crocorum *grown at the temperatures indicated on meat-malt extract agar in moderately deep equal layers in petri dishes.*

(Each figure is an average from seven or eight dishes and the diameter of each colony was measured in two directions at right angles to the nearest $\frac{1}{2}$ mm. $2\frac{1}{2} \times 3$ mm. was deducted for the size of the slab of the original inoculum.)

Temperature	1st period	2nd period	3rd period	4th period	5th period	6th period	Average total increases in diameter of colonies from time of inoculation (see Fig. 2): In 3 weeks	In 4 weeks
7–15·5° C. Av. 11° C.			5·9*	5·8	8·1	8·5	5·9	11·7
19° C.	3·6	11·8	13·6	12·8	11·65	10·45	29·0	41·8
25·5° C.	5·1	15·7	16·9	15·1	9·4	7·35	37·7	52·8
30° C.	4·9	12·8	14·4	12·3	10·75	lost	32·1	44·4
37–40° C.	Nil, after developing for 3–4 weeks at 30° C.						—	—

The results of a second similar set are shown graphically in Fig. 3.

* Total of first three periods.

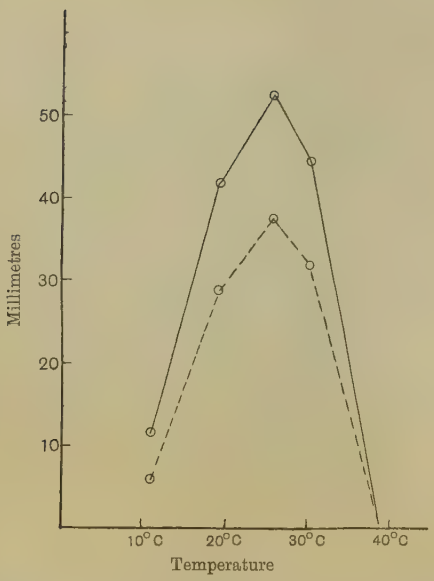


Fig. 2. Graph showing the comparative increase in diameter of colonies of *Rhizoctonia Crocorum* at different temperatures on meat-malt extract agar in three weeks – – – – – – and in four weeks ———— from inoculation. (The temperatures for the values indicated by points at 11° C. and 39° C. are approximate only.)

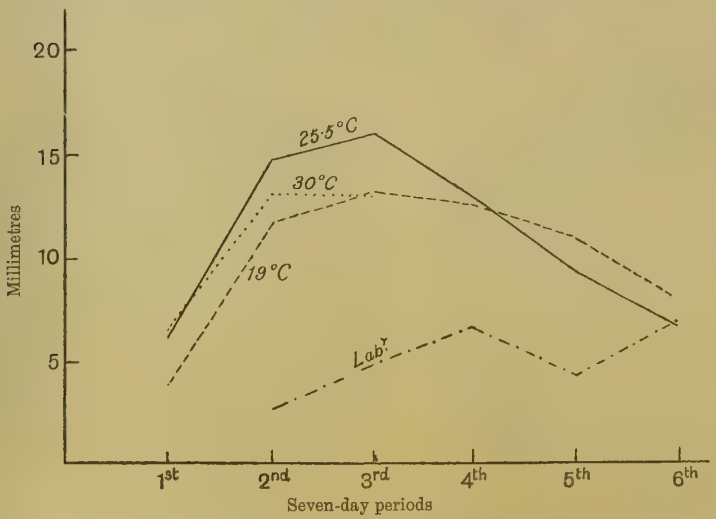


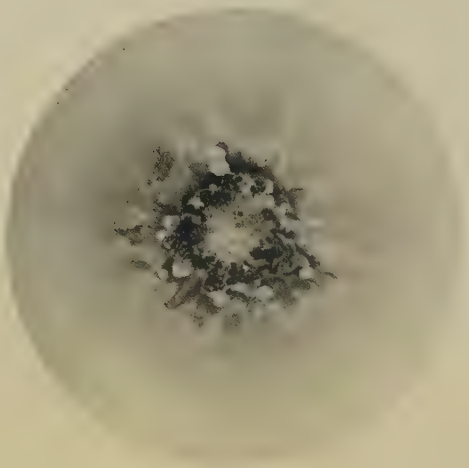
Fig. 3. Graph showing the increase in diameter of colonies of *Rhizoctonia Crocorum* for consecutive seven-day periods from the time of inoculation at the temperatures given. (The readings for 30° C. after three weeks were not obtained in this set.)

Low lab. temp. (av. 11° C.) – • – • – • – • – • 19° C. – – – – – – – – – – – – – –
25·5° C. ———————— 30° C.

Working with his culture contaminated by a *Fusarium* Diehl states: "There was no apparent relation of various temperatures to this culture as exhibited in forty days growth on 'radicicola' agar, Thaxter's potato hard agar, and alfalfa root plugs at 10°, 20°, 22° to 25°, 30° and 37° C." On the other hand, Faris, working with potato plants grown at 90–100° F., obtained good growth of the *R. Crocorum* from infected "sets" whereas plants grown at 60–70° F. did not develop the disease. He states: "there is some indication that the fungus develops best at high temperatures." Our results given above show definitely that the optimum temperature for growth of *R. Crocorum* in culture, as measured by the increase in the diameter of colonies on meat-malt extract agar plates, lies in the neighbourhood of 25·5° C.—possibly a degree or so higher if, as is likely, the fungus shows a smooth temperature-growth curve similar to that given by other organisms of which more complete records can be found in the literature (cf. Fawcett).

Previous writers have attempted to correlate the occurrence of the disease in the field with the probable moisture relations of the fungus. Thus, according to Duggar, du Hamel represented the violet root fungus as selecting dry localities; the Tulasne brothers reported that while wet weather may give the fungus an advantage, it is found in the driest situations permitting crop growth; the fungus occurs very generally in southern Europe, especially in southern France and Italy, and in the United States it has been reported largely from regions of lower humidity and lesser rainfall. The temperature of 25·5° C., which has been shown to be the optimum for active growth of the organism, is high for a field parasite in this country, and, indeed, it is rare to find more than a few plants attacked in very limited areas in the field here. While, no doubt, moisture plays an important part in affecting the activities of the parasite, the authors are of the opinion that temperature is probably the most important factor in governing the relative abundance of the fungus in the various countries from which it has been reported.

The more definite knowledge concerning the temperature relations of *R. Crocorum* which has now been obtained is of importance in that it will allow of a more satisfactory interpretation of the results of cross-inoculation experiments and a sounder determination of the host range of the organism.



(*a*)



(*b*)



(*c*)

BUDDIN & WAKEFIELD.—Some Observations on the Growth of *Rhizoctonia Crocorum* (Pers.) DC. in Pure Culture. (pp. 292—309.)

Summary.

The organism dealt with in this paper is that which has been commonly known in this country as *Rhizoctonia violacea* Tul. but until satisfactory proof is forthcoming of a spore stage of the organism it should be known by its valid prior name, *Rhizoctonia Crocorum* (Pers.) DC.

The methods used for isolation of the fungus in pure culture are described and some account given of preliminary inoculation experiments with the re-isolation of the parasite.

It is shown that growth of *R. Crocorum* will occur on a very wide range of sterilised artificial material when in pure culture.

No spore form has occurred in any of the large number of cultures which have been grown.

Some notes are given on difficulties arising in temperature relation work from differences in the "internal condition" of the organism under varying conditions, and it is shown that the optimum temperature for the growth of the organism in culture on meat-malt extract agar as measured by the increase in diameter of colonies is in the neighbourhood of 25·5° C. This optimum temperature probably explains the geographical distribution of the severe attacks of the organism in the field.

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EXPLANATION OF PLATE X.

Photographs showing the appearance of colonies of *Rhizoctonia Crocorum* after growing for five weeks at (*a*) 19° C., (*b*) 25·5° C., and (*c*) 30° C. (Nat. size.)

Photos by G. Atkinson.

(*Received May 10th,* 1924.)

THE FUNCTION OF LINT HAIRS IN THE LIFE HISTORY OF THE COTTON PLANT

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Considering the importance of raw cotton and the amount of attention it has received from all branches of science it is rather remarkable that a really satisfactory explanation of the natural function of lint hairs has not yet been put forward.

Haberlandt and others consider it as a feeble means of seed dispersal. Denham[1] remarks that no explanation is necessary and makes the unique suggestion that the lint is an outlet for excess polysaccharides. This would be the first instance on record of excess food material being stored in such a manner as to be unavailable for future use.

The lint cannot be of any value to the cultivated plant since it is removed immediately after maturation when it should reasonably be expected to assume its functions. Balls[2] observed in Egypt that self-sown cotton plants showed a better chance of survival than ginned seeds under the same conditions of water shortage. Actual germination tests however show that the seeds germinate quicker after de-linting and give a higher percentage germination. To argue from this that the lint has no useful function to the plant in the natural state shows a lack of appreciation of the peculiar character and adaptation of the cotton plant. Besides it is hardly a comforting thought that such an important industry rests on a biological freak.

The specialised types of cotton possessing high yield of lint of long staple (e.g. *Gossypium barbardense*) are annuals of rapid growth and early maturity and appear to grow best in a climate of well-marked wet and dry seasons. In other words, their natural habitat is one requiring xerophytic characters, yet the cotton plant is remarkably free from such characters and is exceedingly sensitive to soil moisture conditions and solar radiations. Under cultivation the crop is sown towards the end of the rainy season and as the dry season advances a very extensive root

[1] *Shirley Institute Memoirs*, I, 1922.
[2] *Development and Properties of Raw Cotton*, 1915.

system is rapidly developed to keep in touch with the receding water table. Any subsequent abnormal rise in the water table is attended with damage to the crop.

In a wild state the seeds would require protection from temporary changes in humidity such as might occur with heavy dew or sporadic rainfall that might initiate germination without provision for subsequent growth. For this purpose a thick covering of cotton lint is eminently suitable. Also the lint being rolled about by the wind during the dry season, would collect round it the débris of dead leaves, etc., and ultimately come to rest in some hollow or sheltered position. With the advent of the definite rainy season humification of the mass of lint and débris would have set in before germination occurred.

Thus the evolution of lint hairs may be considered a special adaptation to climatic conditions. Even to-day, notwithstanding the artificial extension of cotton areas, the varieties with most highly developed lint (*e.g.* Sea Island and Egyptian) are to be found only in climates with well-marked regular seasons whilst the types with more fuzzy types of lint are found in more erratic climates.

(*Received April 2nd*, 1924.)

STUDIES IN BACTERIOSIS

XI. A BACTERIAL DISEASE OF LETTUCE

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(With 2 Text-figures.)

This disease of lettuce occurred during the summer of 1921 and the extent of the damage caused is not known; the material was submitted for examination by the Ministry of Agriculture and Fisheries. It came from a private garden where 75 per cent. of the plants were attacked. The disease shows itself by a browning of the leaves beginning usually at the margins and working inwards; the veins are first attacked and appear as a fine brown network; the attacked tissue becomes the prey of saprophytic bacteria and moulds and is quickly reduced to a wet rotting mass. From portions of the leaf in which only the veins were attacked an organism was isolated and obtained in pure culture without much difficulty. This organism produced the disease when deposited on the surface of healthy lettuce either by spray or brush inoculation. A photograph of the original diseased plant appears in Fig. 1 and the result of one of the infection experiments is shown in Fig. 2.

Description of the Causal Organism.

A. *Morphological Characters.*

The organism is a bacterium with polar flagella, one to three at one pole (a few cases were noted with five at one pole). Caesares-Gil's Flagella stain was used to demonstrate the flagella. The organism is a short rod with rounded ends occurring singly or in chains.

Stained with carbol fuchsin the organism measures 1·6 μ to 2·2 μ long and 0·8 μ to 1·1 μ wide, the average size being 2 μ long by 0·9 μ wide.

Stained with gentian violet the organism measures 2 μ to 1·3 μ long by 1 μ to 0·5 μ wide, the average size being 1·6 μ long by 0·8 μ wide.

The staining and measuring were carried out on 18–24 hour old cultures on bouillon agar.

Capsules were suspected, and eventually a successful preparation showing them was made with Ribbert's capsule stain.

Endospores were not observed. Tests for spores were made by heating old bouillon cultures of various ages to 80° C. for 20 minutes. Transfers were made in each case before and after heating, but no growth was obtained in cultures made by transfers after such treatment, although before heating the cultures were alive.



Fig. 1. Showing a diseased lettuce plant. Fig. 2. Showing the result of inoculation.

Behaviour towards Stains. The organism stains readily with carbol fuchsin, gentian violet, methylene blue, victoria blue and bismarck brown. It is Gram negative and not acid-fast.

B. *Cultural Characters.*

The organism grows readily in most media, but growth is most rapid in bouillon (*p*H 6·8–7) at 30° C.

In the following series of experiments the temperature of incubation was 25° C. unless otherwise stated.

B. Agar Plates (*p*H 6·7). The colonies on poured plates appear after 24–28 hours. Surface colonies are 1½–2 mm. in diameter after two days, later they vary in size from 2–5 mm. Depth colonies are lenticular, and vary from 0·5–1 mm. in diameter. The surface colonies are round, convex and wet-shining. The colour is ivory-yellow[1] by reflected light, and cartridge-buff by transmitted light. The medium is at no time stained by the organism. Microscopically the colonies appear granular and somewhat denser at the centre.

Streak Culture on B. Agar (*p*H 6·8). Growth is visible after 24 hours. In three days the growth is thin, spreading, wet-shining, and ivory-yellow in colour. No staining of the medium is visible. The culture smells decidedly, the smell resembling that of rotting cabbage.

Stab Culture in B. Agar (*p*H 6·8). Growth is visible on the surface after two days, the colony being 4 mm. in diameter. After eleven days, the surface colony is 1 cm. in diameter, and is flat, thin, entire and of a cream-buff colour. The medium is not stained. Growth is limited to the surface.

Plate Culture on B. Gelatine (*p*H 6·8). Colonies become visible in 24 hours at air temperature, and the gelatine is partially liquefied. In three days the plate is entirely liquefied. The colonies are 1 mm. in diameter.

Stab Culture in B. Gelatine (*p*H 6·8). Liquefaction begins as a crateriform depression in 1–2 days at air temperature, and there is slight growth visible in the upper part of the stab. In eleven days, the liquefied portion of the medium is 7 mm. deep, stratiform, with a heavy precipitate. After six weeks, the gelatine is not entirely liquefied, the solid portion occupying about 1·5–2 cm. at the bottom of the tube.

Streak Culture on Cooked Potato. Growth is visible after 24 hours. The streak is thin, spreading, and honey-yellow in colour; in eleven days, the potato medium has darkened slightly.

Nutrient Bouillon Broth (*p*H 6·8). Strong clouding of the medium takes place in 16–24 hours at 25–30° C. After eleven days, the medium is persistently turbid, with a compact sediment which is viscid on agitation; the medium is naphthalene-yellow in colour with very slight fluorescence; the tubes have a decided odour, which resembles rotting cabbage.

[1] The colours mentioned in this paper are given according to Ridgway (Ridgway, Robert, *Color Standards and Color Nomenclature*, 43 pp., Col. Pl. Washington, D.C., 1912).

C. *Physiological Characters.*

For the following tests a 48-hour old culture in bouillon was used for inoculating the tubes, which were afterwards incubated at air temperature (15–20° C.).

1 *per cent. Glucose and Peptone Broth.* Growth is visible after 24 hours, and the litmus shows an acid colour in two days, and there is a slight pellicle. After four weeks the medium is onion-skin-pink in colour with a congo-pink precipitate; no gas is formed, and no clouding takes place in the closed limb of the Einhorn tube.

1 *per cent. Lactose and Peptone Broth.* Growth is visible in two days, but no change in the colour of the medium takes place; a slight pellicle is formed after eleven days; there is no change in appearance of the tube at the end of two months; no gas is formed, and no growth takes place in the closed limb of the tube.

1 *per cent. Sucrose and Peptone Broth.* Growth is visible after two days and in four days the litmus shows an acid reaction in the upper part of the tube; after four weeks there is a corinthian-red pellicle and the upper part of the medium is salmon coloured, while the lower part is bleached; there is no gas formation, and no growth takes place in the closed limb of the tube.

1 *per cent. Maltose and Peptone Broth.* There is clouding and a slight pellicle after eleven days; after six weeks the tube shows no colour change, and no gas is formed.

1 *per cent. Mannite and Peptone Broth.* There is clouding of the medium after eleven days, but no change in colour of the medium is visible; after six weeks the tube shows no change, and no gas is formed.

1 *per cent. Peptone Broth.* The same result as with mannite above.

1 *per cent. Nitrate Broth.* Growth is visible in the tube after four days. There is no reduction; tests for nitrite using α-naphthylamine-sulphanilic acid were made weekly for six weeks and consistently gave negative results.

Potato Plug. About 1 c.c. volume of plug was taken and ground up in 200 c.c. of water. The addition of 1 c.c. of iodine solution produced a blue violet, which was not so intense in colour as in the case of the control plug but the difference was not sufficiently marked to indicate diastatic action.

Uschinsky's Solution. The medium is turbid on the second day; in eleven days there is a heavy pellicle with long filaments hanging down into

the medium, which shows fluorescence and is light-lumière-green in colour, and very turbid.

Cohn's Solution. There is no growth in this medium; at the end of six months the medium in the tube is quite clear.

Litmus Milk. A slight reddening of the litmus in the top layer of the tube is visible in two days; this reddening gradually spreads downwards and after eleven days has reached the bottom of the tube; by this time the top centimetre of the medium is clear, but a reddish ring persists on the sides of the tube at the surface of the medium. After a month, the medium is entirely bleached, the upper two-thirds being clear, while the curd occupies the lower third.

Indol Production. Tests for indol were made by means of the Rosindol method, but negative results were obtained in all trials.

Temperature Relations. The Thermal Death Point lies between 51° and 52·5° C. The maximum temperature for growth is below 37° C. A bouillon culture kept at 37° C. for seven days shows no growth, but becomes turbid after 24 hours when transferred to the 25° C. incubator. The minimum temperature for growth is below 10° C. The lowest limit was not ascertained. The optimum temperature is 30° C.

Relation to Moisture. The organism is not very sensitive to drying. Loop transfers were made from a 19-hour old bouillon culture on to sterile strips of mica, kept at air temperature in the dark in a ventilated Petri dish. One of these strips was transferred daily to a tube of bouillon in which it was shaken up. These daily transfers were continued for 14 days in succession and in each case growth occurred in the bouillon tube after incubation for seven days at 25° C.

Vitality in Culture Media. The organism lives for six months and more in bouillon kept at room temperature (15–20° C.).

Group Number. According to the older descriptive chart of the Society of American Bacteriologists the group number of this organism is 211. 2322133.

Relationship of the Causal Organism[1].

From the first series of cultural experiments, the organism described in the foregoing pages was suspected of identity with, or near relationship to, *Bacterium marginale*, an organism which was first isolated and described by Miss Nellie A. Brown in America(3). To gain further in-

[1] Since this paper was sent to press Dr Mehta and Dr Berridge have shown (see the following paper, p. 318) that *B. marginale* is identical with *B. pyocyaneus*, and there is no doubt that the organism here described is also a strain of this latter organism.

formation on this point, application was made to Miss Brown for a culture of *B. marginale.*

Miss Brown kindly supplied cultures of three organisms pathogenic to *Lactuca sativa*, all isolated and described by her. With these cultures, a parallel series of cultures of all four organisms was set up and carefully watched for six weeks. It was soon evident that *Bacterium marginale* and our organism were most nearly allied, and no further comparative study was made with the organisms *Bacterium viridilividum* and *Bacterium vitians*. Henceforth parallel experiments were confined to *Bact. marginale* and our organism. From these experiments it appears that although the organisms have many reactions in common, they are not identical.

It was noticed that the "Group Number" for our organism agreed with that given for *Bacterium aptatum*, isolated and described by Miss N. A. Brown and Miss C. O. Jamieson as pathogenic to Sugar-beet and Nasturtium leaves(1) and also with that given for *Bact. xanthochlorum* and *Ps. tenuis*. Application was made to Miss Brown for cultures of these organisms, but she was only able to supply a culture of *Bact. aptatum*. On receipt of this culture a comparative series of cultures of our organism and *Bact. aptatum* was set up. From these tests it was found that the two organisms were in essential characteristics so nearly identical that in the opinion of the writers the two are one and the same species. Any slight differences which exist may be attributed to adaptation to different hosts.

The authors desire to take this opportunity of expressing their thanks to Miss N. A. Brown for her courtesy in supplying cultures and information which have been used in this work.

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(*Received May 11th*, 1924.)

STUDIES IN BACTERIOSIS

XII. *B. PYOCYANEUS* AS A CAUSE OF DISEASE IN LETTUCE AND THE IDENTITY OF *B. MARGINALE* WITH THIS ORGANISM

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(With Plate XI.)

In 1918 a description of two species of bacteria which attack lettuces, and had been the cause of considerable loss to market gardeners in America, was published by Miss N. Brown (1). Sub-cultures of these were received in 1922 by Dr S. G. Paine from Miss Brown for purposes of comparison with an organism isolated from diseased lettuces grown in England.

One of these organisms, *B. marginale*, has, since then, been kept in this laboratory, mainly on bouillon agar, and has been regarded as a typical plant parasite of the Pseudomonas type. Its power to produce green coloration of the medium has become more and more marked, so much so as to suggest the possible identity of this organism with *B. pyocyaneus*.

One of us (M. M.) had previously been engaged in a careful redetermination of the morphological and cultural characters of a series of bacterial species; these included *B. marginale* and a strain of *B. pyocyaneus* obtained from the Lister Institute. When the records for these were compared they were found to be identical in every respect and are given in Table I.

These redeterminations of the characters also correspond very closely with those of Miss Brown's original description of the species, proving that it had not altered materially in the course of the two years it had been kept in this laboratory. One or two discrepancies certainly are to be found; for instance, Miss Brown states that *B. marginale* produces acid in saccharose after six weeks, whereas we concluded that no acid was formed since our cultures in saccharose rapidly became green and

remained so for three months, and this green colour is in itself an indication of the alkalinity of the medium.

The characters given in Table I also agree with those given by Lehmann and Neumann (2) for *B. pyocyaneus*, except that our strain of the organism was Gram-negative, as was also *B. marginale*. Tromsdorf (3), however, who studied 27 strains of *B. pyocyaneus*, found that some strains of the organism were Gram-negative.

Table I.

Showing the close agreement of B. marginale *and* B. pyocyaneus *in their morphological and cultural characters.*

	B. marginale	*B. pyocyaneus*
Spores	None	
Aerobism	Markedly aerobic ? Facultative anaerobe—very slight growth observed	
Liquefaction of gelatine	Rather slow liquefaction	
Glucose broth	Acid, no gas, no green colour	
Lactose broth	No acid, no gas, peacock-green colour Ring and pellicle on the surface on the 3rd day	
Saccharose broth	No acid, no gas, peacock-green colour Ring and pellicle on the surface on the 3rd day	
Nitrate broth	Good growth, much gas after 24 hours Strong reduction, nitrate and nitrite has all disappeared by the 15th day	
Chromogenesis	Yellow-green turning to bluish-green as the alkalinity of the medium increases	
Growth on potato	Greenish-buff in 48 hours	Sometimes yellow, sometimes greenish-buff in 48 hours
Diastase reaction	Very slight, if any	
Morphology	Very slender rods, rounded at the ends, actively motile by one polar flagellum, capsulated	
Size	2·4–1·5μ × 0·6μ	2·7–1·5μ × 0·5μ
Gram-stain	Negative	
Thermal death point	54° C.	
Growth in broth	Good growth with yellow-green colour in 24 hours at 25° C., pellicle formed in 48 hours	Good growth with green colour in 24 hours at 25° C., pellicle on the 3rd day
Peptone water	Clouding with light blue colour in 36 hours, bluish-green colour on the 3rd day	Clouding with light green colour in 36 hours, dark green colour on 5th day turning salmon-pink after a fortnight
Indol	Could not be determined, as any acid, *e.g.* a few drops of dilute hydrochloric acid, gives a pink colour to the culture	
B. agar streak	Dull yellow growth in 18 hours, medium turns green in 36 hours, and the growth shows a pearly lustre	Dull greenish moist growth with "erose" margin in 36 hours, medium turns green on the 4th day, and the growth shows a pearly lustre
Bouillon agar plate	Small greyish-white colonies in 48 hours. Medium turns bluish-green by the 3rd day	Small round yellowish-white colonies in 18 hours. Medium turns from yellowish to bluish-green by the 9th day

Table I (*contd.*).

	B. marginale	*B. pyocyaneus*
Gelatine plate	Tiny greenish-white irregular colonies in 48 hours, with cup-shaped depressions due to liquefaction. On the 3rd day general liquefaction occurs, and the medium turns dark green	Tiny greyish-white irregular colonies with granular appearance in 18 hours, with green coloration of the medium. Slight liquefaction by the colonies on the 2nd day which becomes general on the 3rd day
Gelatine stab	Filiform growth in 36 hours, stratiform liquefaction in 5 days, green colour at the surface	Cylindrical growth in 36 hours, stratiform liquefaction in 10 days, green colour at the surface
Milk	Alkaline in 54 hours; a soft clot is formed, and peptonisation begins on the 7th day. A greenish curd persists for at least 2 months	Alkaline in 54 hours; a soft clot is formed, peptonisation begins on the 3rd day. A curd persists for at least 6 weeks, and the liquid above turns green
Uschinsky's solution	Alkaline, no gas, bluish-green ring and pellicle in 18 hours, yellow-green colour on the 3rd day	

Infection experiments.

Healthy young lettuce leaves, and plants of the Cos variety from which the outer leaves had been removed, were sprayed with 24-hour cultures of *B. marginale* and *B. pyocyaneus* in "Jardox" peptone broth, and also with cultures in the same broth with 1·5 per cent. glucose added. The cultures were diluted with sterile water before spraying, and were boiled for half-an-hour before spraying the controls.

The disease first appeared as minute rust brown spots along and near the midrib, veins and margins of the leaves, particularly in the pockets where the culture suspension had lodged. The organisms evidently enter by the stomata, the cells surrounding them becoming filled with a brownish pink zoogloea, the pink colour being characteristic of *B. pyocyaneus* when growing in an acid medium. As the spots spread and coalesce, brown patches appear, particularly along the margins, and later the whole leaf becomes brown and flaccid. To obtain photographs of them in this condition (Plate XI, fig. 4) they had to be floated in water on to sheets of glass as they would not bear touching. The midrib turns a rust brown colour and later also becomes soft and pulpy.

The following table gives details of the course of the disease due to *B. pyocyaneus*, and shows that the symptoms are essentially the same as in the case of disease due to *B. marginale*; the only difference being time variations, and even these are not constant.

Lettuce Leaves.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
I. Bouillon	4	Brown patches at the apex	A few spots at the apex
	6	Brown patches all over the leaf, midrib brown, margin dead	One patch at the apex
	8	Completely withered and dead	Right half of leaf dead
	9	—	Completely withered and dead
Control with boiled broth	9	Healthy except for slight damage at the apex	Healthy
II. 1·5 % glucose broth	5	Healthy	Many tiny brown spots at apex
	10	Large brown patches on margin and lamina	Apex and margin yellow
	13	Lamina almost completely withered and dead	One half of lamina almost completely dead
	15	Dead	Dead
Control with boiled glucose broth	15	One leaf healthy. Second leaf healthy till 10th day, then fungus spot appeared at apex	One leaf healthy. Second leaf slightly spotted after the 8th day

These experiments were repeated three times with similar results.

Lettuce Hearts.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
I. Bouillon	3	Many small brown patches	No spots
	4	One leaf dead, others showing brown patches	Margin at apex brown
	6	Four leaves dead	Leaves brown and dead at the apices and along the margins
	8	Whole withered and dead	Upper halves of leaves dead
	10	—	Whole withered and dead
II. 1·5 % glucose broth	3	Healthy	Apices and margins spotted and turning brown
	4	A few brown spots at apex of leaves	Brown patches at apex of leaves
	6	Many spots	Upper part of three outer leaves dead
	8	Upper part of outer leaves dead	Upper half dead
	10	Whole dead	Whole dead
III. Control boiled glucose broth	12	Healthy, apex has grown 2–3 inches	Healthy

These experiments were repeated with similar results.

Midribs of Leaves.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
1·5 % glucose broth	4	Centre turning brown	Rot beginning on one side
	9	Rot slowly spreading	Rot spreading
	14	Rot still spreading	Quite rotten
Control with the same broth boiled	14	Quite healthy	Quite healthy

These experiments were repeated with similar results.

Stems.

Although stems were repeatedly inoculated with broad organisms, and the infection pricks in a few cases became brown and exuded a watery fluid, no general infection of the tissue followed.

Re-isolation of B. pyocyaneus *and* B. marginale.

A portion of a leaf which had turned brown four days after spraying with *B. pyocyaneus* was ground up with sterile sand and plated on agar. Pure cultures of smooth, round, yellowish-white transparent colonies were obtained on the following day, which later developed the green pigment, pearly iridescent lustre and umbonate form characteristic of the species. Inoculation of the crushed leaf into broth also gave the green colour.

B. marginale was re-isolated in the same manner from a leaf showing light brown patches nine days after spraying with a glucose broth culture of this organism. The colonies on agar plates resembled those of *B. pyocyaneus* in every respect.

Both cultures were carefully re-tested, especially for acid production in sugars. Both again gave no acid with saccharose, and the green colour appeared on the 4th day, while with glucose they gave no acid reaction, and up to the 20th day no green colour had developed.

In nitrate broth reduction to nitrite had already begun on the 2nd day, and was complete by the 15th.

The striking series of colour changes in litmus milk described by Miss Brown for *B. marginale* also occurred in the milk cultures of both organisms; they showed on the 2nd day a pink ring merging into a yellow and white pellicle while the rest of the milk below was a lilac litmus colour. Digestion began on the 3rd day, and by the 7th a green ring had appeared below the yellow pellicle and a soft pinkish curd had separated at the bottom of the tube.

It seems rather significant that this disease of lettuces occurred in America at greenhouse temperatures, on highly manured soil, and after the plants had been splashed and flooded with water. *B. pyocyaneus* is an organism frequently found in water, especially in the tropics, and is an intestinal organism able to grow vigorously at body temperatures.

Summary.

B. marginale, the causal organism of a disease of lettuces described by Miss N. Brown in 1918, appears to be identical with the well-known



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

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B. pyocyaneus. They agree in their morphological and cultural characteristics, and the latter has been shown to be capable of attacking young lettuce leaves and producing disease similar to that due to *B. marginale*.

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EXPLANATION OF PLATE XI.

Fig. 1. Heart of diseased lettuce eight days after inoculation with *B. marginale*; tissues blackened.

Fig. 2. Heart of diseased lettuce ten days after inoculation with *B. pyocyaneus*; tissues blackened.

Fig. 3. Heart of a lettuce used as control; quite healthy ten days after spraying with a boiled culture.

Fig. 4. Lettuce leaves; that on the left sprayed with *B. marginale*, that on the right with *B. pyocyaneus*. Both show spots and withered brown margin.

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ON THE STIMULATING ACTION OF CERTAIN ORGANIC COMPOUNDS ON CELLULOSE DECOMPOSITION BY MEANS OF A NEW AEROBIC MICRO-ORGANISM THAT ATTACKS BOTH CELLULOSE AND AGAR

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(With Plates XII and XIII and 2 Text-figures.)

The decomposition in the soil of plant residues, which are largely composed of cellulosic material, is of great importance to agriculture, and the nature of this process is at present but little understood either from its biological or its chemical aspect. Cellulose is decomposed with some difficulty and by but a limited number of organisms; the products of its decomposition appear, however, to be of great value in the economy of the soil. For example, under proper conditions of aeration, moisture, etc., crop residues give rise in the soil to the humic matter which plays a large part in crop production. Although the steps leading to the formation of this humus are at present not clearly defined, there is reason to think that further knowledge of the decomposition of cellulose under such conditions as obtain in the soil will throw light on this important question.

The earliest biological investigation, made by Omeliansky, dealt with the breaking down of cellulose by anaerobic bacteria. In normal arable soils, however, the conditions are largely aerobic, and, while the work of Omeliansky and Solunskoff(10), and of Truffaut and Bezssonoff(14), on nitrogen fixation by *Clostridium Pasteurianum* suggests the possibility that symbiosis between aerobic and anaerobic bacteria may be a factor to be considered, it seems to be of first importance that knowledge should be obtained as to the aerobic soil organisms capable of attacking cellulose, and the conditions under which they operate.

Bacteria that can decompose cellulose under aerobic conditions are known to occur fairly extensively in nature. Van Iterson(6) was the first to draw attention to this type of organism; he did not, however,

obtain a pure culture of the bacteria which brought about the destruction of the cellulose. Several species have been described by Kellerman and McBeth(7) and their associates(8,9); these workers lay especial stress on their finding that these organisms rapidly lose the power of destroying cellulose when grown under cultural conditions. Their criterion for cellulose destruction lay in the appearance of a clear zone about colonies on agar media which contained precipitated filter paper as the source of carbon. Hutchinson and Clayton published in 1919 their paper(4) describing a spirochaete-like organism to which they gave the name *Spirochaeta cytophaga*. This organism does not grow on the ordinary media containing organic substances such as peptone and meat-extract, and great difficulty was experienced in obtaining pure cultures until a dilution method was adopted. Cultures were then grown successfully on agar enclosing sheets of filter paper, and cultivated in tubes of liquid media consisting of a strip of filter paper half immersed in mineral salts solution. This appears to us to be a better criterion of cellulose destruction than that of Kellerman and McBeth, for a great many soil organisms can grow on plain mineral salts agar without any other source of energy material. Moreover, the appearance of a clear zone is sometimes produced around colonies owing to the solution of phosphates and carbonates in the medium.

In the course of purifying cultures to isolate *Sp. cytophaga*, a new type of aerobic cellulose-decomposing organism was obtained. A description of this new organism follows.

Part I.

MICROSPIRA AGAR-LIQUEFACIENS (n.sp.).

The new organism isolated has the following characters:

Source. Rothamsted soil, 9th June, 1923.

Morphology. Short curved rods, usually C-shaped, with occasional S-shaped and coccoid forms.

Size. 2μ long by 0·5μ to 0·7μ broad; 3μ to 5μ long in division stages. Coccoid forms difficult to measure in the living state, but stained are 0·5μ to 0·7μ long.

Motility. Very active in young cultures. Cells remain active for some months.

Flagella. Single, polar; short, as long as or twice the length of the major axis of single cells; slightly undulating.

Oxygen Relations. Very aerobic. No growth takes place in the

closed arm of the fermentation tube. In liquid cultures containing filter paper it first attacks the paper at the air-liquid level.

Temperature. Optimum 25° C. Will grow at 16° but not 34°.

Agar Slope. Peptone (0·5 per cent.), Lemco (0·3 per cent.), Agar (1·5 per cent.).

In 24 hours at 25° C. a deep groove is cut along the inoculation streak, a whitish growth lining the sides. As growth reaches to its maximum the groove becomes deeper and the liquid at the bottom increases to more than twice its volume (Plate XII, fig. 2). The middle of the slope bulges outward on account of the gel being weakened. Old cultures, on being shaken, break down to amorphous lumps of agar.

1 per cent. glucose added to nutrient agar prevents the liquefaction from taking place.

Agar Colonies. The same medium as above.

Five days at 25° C. *Surface colonies.* Whitish growth in a depression, surrounded by a white ring. Under the half-inch objective; a brown central nucleus shading off to transparency, surrounded by a wide greyish band with an undulating inner and entire outer edge. These characters are not always visible on account of the variability in the amount of gel-weakening. Motile organisms can sometimes be seen around the outer edge, pushing their way into the zone of semi-liquefied agar. In colonies of this age the agar film is entirely eaten away within the area of the colony, the growth made by the organisms resting on the bottom of the dish (Plate XII, fig. 3). The colony is surrounded by a zone of about a quarter of an inch in width of semi-liquefied agar, which shows as a clear ring. In older colonies, about 14 days at room temperature (16–18°), the centre of the colony begins to grow convex with a smooth surface, surrounded by a bluish area of semi-liquid gel. At four to five weeks no further liquefaction has taken place, and the colonies have become irregular with a rugose surface having a resinous appearance.

Deep colonies show a clear area of similar width. They may be irregular, oval, or angular.

Agar Stab. Same medium as above.

A deep hollow is formed on the surface, about 3 mm. deep, in 4–5 days at 25° C. After a month or more the whole surface is liquefied, the liquefaction extending down the sides against the glass and the unliquefied gel having a convex surface.

From a young inoculum a hollow is formed within 24 hours.

Gelatine Stab. Very slight surface growth after one month; the

streak then shows a beaded line. There is no liquefaction. The beading is less pronounced if the stab is made from a young inoculum.

Starch Agar. Of the same composition as the above agar, with the addition of 0·1 per cent. of Lintner's soluble starch. The agar is poured into sterile petri dishes and allowed to harden. When cool, the surface is streaked with the inoculum, and incubated at 25° C. In 48 hours or less a groove appears along the surface and, as in the case of the agar slope, the agar is consumed. Diastatic action is tested for by flooding the plate with a saturated solution of iodine in 50 per cent. alcohol.

3 days	7 days
Diastatic action pronounced	Stronger, 2 cm. diam.

With the most viable inoculum liquefaction begins in 18 hours.

Nutrient Broth. Peptone (0·5 per cent.), Lemco (0·3 per cent.). No scum or sediment. Slightly cloudy liquid.

Carbohydrate Broth. Composed of 1 per cent. peptone and 1 per cent. of each carbohydrate as under, incubated for seven days at 25° C. Duplicate test tube cultures were inoculated in each case, and tested with five drops of neutral litmus solution.

Action on Carbohydrates.

Carbohydrate	Growth	Acid in
Dextrose	Slight	Both
Sucrose	Slight	Neither
Lactose	Slight	Both
Maltose	Slight	Both
Glycerine	Nil	Neither

Nitrate Broth. The development of acidity, where present, was slight.

Peptone (1 %), KNO_3 (0·1 %)	No growth.
Nutrient broth with KNO_3 (0·1 %) ...	No nitrite at 7 days. Nitrite at 14 days.

Cellulose Media. Filter paper medium, composed of a strip of filter paper half immersed in mineral salts solution of the following composition.

KNO_3	0·1 %	NaCl ...	0·01 %
K_2HPO_4	0·1	$CaCl_2$...	0·01
$MgSO_4.7H_2O$...	0·02	$FeCl_3$...	0·002

The paper undergoes rapid disintegration. After two or three days incubation at 25° C. fibres of the paper are thrown down from the level at which the paper is being destroyed. The paper then breaks in two and

the two edges show very ragged outlines, taking on a pulpy consistency (Plate XII, fig. 1). After a month or more in this medium the paper becomes a pulpy mass at the bottom of the tube, but large areas of the paper appear not to have been attacked.

The nitrate in this medium is rapidly reduced to nitrite, as is shown by testing with α-naphthylamine-sulphanilic acid. After two months the red colour given by this reagent is faint, and on testing a 4 months old culture no trace of the nitrite can be observed. The solution remains on the alkaline side of neutrality. No ammonia is produced and the whole of the nitrate is utilised.

With $(NH_4)_2SO_4$ 0·1 per cent. or $NaNO_3$ 0·2 per cent. as the source of N the organism grows equally well. Less rapid growth takes place if nitrogen is not added; the amount of organic N present in this filter paper (0·018 per cent.) apparently being available to the organism. The paper is broken through in 14 days time. A square of filter paper wholly immersed in the solution is attacked much less rapidly than where the paper is half immersed.

Cellulose agar, made according to the directions of Kellerman (7, 8). No growth occurred on plates or slopes.

Cellulose sheet agar, consisting of a sterile sheet of filter paper applied to the surface of mineral salts agar and dilute suspensions of the organisms in salts agar poured on in a thin layer (Hutchinson's method for *Sp. cytophaga*). No colonies appeared, nor was the gel liquefied.

Cellulose sheet agar, a sterile strip of filter paper applied to the surface of a mineral salts agar slope was streaked with a culture of the organism. It grew well in the liquid at the bottom but not on the surface of the paper.

Method of Attacking Filter Paper. The organism apparently enters the fibres and multiplies in the central canal; in young filter paper cultures cells may be seen in the living state actively motile within the fibre. In old cultures motility has ceased and the organisms have multiplied to a considerable extent, taking on the coccoid form. These two stages are illustrated on Plate XIII, figs. 2 and 3.

Plain Agar Media. 1·5 per cent. agar with mineral salts as given above but with various sources of nitrogen. Slope cultures were made.

1.	$(NH_4)_2SO_4$	0·1 %	Rapid liquefaction, exceeding that on nutrient agar.
2.	,,	0·06 %	Less rapid than with 0·1 %.
3.	$NaNO_2$	0·1 %	No growth took place.
4.	$NaNO_3$	0·2 %	Liquefied, but less growth than on 2.

5. Nitrogen-free — Slight liquefaction.
6. Nitrogen-free
 Mannitol 1·0 % Slight liquefaction.

The last medium is one on which *Azotobacter* grows well. The amount of organic nitrogen present in the agar used has been found to be as high as 0·272 %, but apparently very little of this is available to the organism.

Viability. Cultures rapidly lose viability in all the media so far tested. This is due apparently to the production of some substance toxic to the majority of cells present, for very few colonies appear on plates even from a thick suspension; this is especially true of cultures a week old or older. Viability is, however, kept by some cells for as long as seven months. On the other hand, the cellulose decomposing power has not been found to decrease after the organism has been sub-cultured for nine nonths.

Systematic Position. On account of its morphological character, coupled with its remarkable power of liquefying agar-gels, the name *Microspira agar-liquefaciens* is suggested for the new organism described. A preliminary note was read soon after its discovery to a meeting of the Pathological Society of Great Britain and Ireland held at Oxford on July 13th, 1923.

The literature gives three references to organisms having the agar-liquefying power. *Bacillus gelaticus*, obtained by Gran from sea water (3), was used in conjunction with *Clostridium Americanum* and with *Azotobacter chroococcum* by E. and H. Pringsheim (12) to supply these nitrogen fixing organisms with a source of energy through the hydrolysis of agar. Panek described in 1905 (11) an organism from sugar-beet under the name of *Bacterium betae viscosum*. In 1911 Biernacki (1) isolated from raisins a facultative anaerobe to which he gave the name *Bacterium Nenckii*. None of these organisms has apparently been tested for the power of decomposing cellulose. They are all, however, considerably different in other respects from the organism here described; we are justified therefore in considering it a hitherto undescribed species.

Part II.

THE DECOMPOSITION OF CELLULOSE BY *M. AGAR-LIQUEFACIENS* IN THE PRESENCE OF SUGARS AND RELATED COMPOUNDS.

As a result of their work on the breaking down of long straw in the presence of soluble nitrogen compounds, Hutchinson and Richards (5) have developed a method by which straw and other materials rich in

cellulose can be decomposed to produce a substance closely resembling farmyard manure of high fertilising value(13). Since the supplies of natural stable manure are becoming less abundant, and since, in many arable areas, farmyard manure is scarce or unobtainable, such methods of producing an organic manure by artificial means must have a great future importance in maintaining the humus content of arable soils.

In the rotting of straw, whether in farmyard manure or in the presence of artificial nitrogen compounds, the disappearance of the cellulose is only one of the processes to be considered and is intimately bound up with the bacterial decomposition of the pentosans, *i.e.* carbohydrates composed of 5-carbon atom groups, which form a considerable proportion (about 50 per cent.) of the carbonaceous matter of the straw. Mr E. H. Richards, in discussing the question of cellulose decomposition with the present authors, suggested the possibility that this decomposition, in materials such as straw, may be modified by the presence of these other carbonaceous compounds. With this possibility in view it was decided to investigate the effect of small amounts of certain other compounds on the rate at which cellulose is decomposed in pure culture. For the purpose of this work it was considered that *Microspira agarliquefaciens* was an organism eminently suitable, owing to the rapidity with which it attacks pure filter paper and its ability to live in the presence of sugars.

The carbohydrates and related compounds selected for these investigations were certain of those that are liable to occur in association with cellulose under natural conditions.

These were arabinose, xylose, glucose, sucrose, starch, dextrin, straw gum, lignin, mannitol. The cellulose used in the experiments was Whatman's No. 41 ash-free filter paper. The mineral salts solution had the following composition:

$NaNO_3$	2·5 gm.	$CaCl_2$	0·1 gm.
K_2HPO_4	1·0	$FeCl_3$	0·02
$MgSO_4.7H_2O$...	0·3	Distilled water ...	1000 c.c.
NaCl	0·1		

To determine the amount of cellulose consumed it was decided to use Schweitzer's reagent. Preliminary trials of the method of estimating cellulose by means of this reagent are tabulated below. It has been thought useful, however, to give first the main details of the preparation of the reagent, as advised by Charpentier(2).

200 gms. of $CuSO_4 . 10H_2O$ were dissolved in boiling distilled water, and a solution of ammonium chloride in sufficient quantity to satisfy

the equation below, followed by excess of a 10–20 per cent. solution of NaOH, were added.

$$CuSO_4 + 2NH_4Cl + 2NaOH = Cu(OH)_2 + 2NaCl + (NH_4)_2SO_4$$

The $Cu(OH)_2$ thus precipitated was washed free from the associated salts and then dissolved in ammonia of ·92 sp. gr., so that by the end of 24 hours nearly the whole of the $Cu(OH)_2$ was in solution.

Whatman's No. 41 filter paper was dried in the steam oven and weighed. It was then dissolved in 100 c.c. of the Schweitzer's reagent, the mixture being stirred with a glass rod, and the solution allowed to stand for one hour. The cellulose was then precipitated by adding just sufficient of a mixture of 100 c.c. concentrated HCl and 400 c.c. H_2O, and, when the blue colour had gone, precipitation was held to be complete. The precipitate was caught by filtering through a weighed filter paper, and washed with a dilute (about 10 per cent.) solution of HCl, followed by hot water until neutral, to remove any traces of $Cu^{\cdot}$ or $Cl^{\cdot}$. The filter paper containing the cellulose precipitate was then dried in the steam oven. The results of this are shown below.

Weights in grammes.

No.	Filter paper (at start)	Cellulose recovered	Loss	Average
1	0·6475	0·6470	0·0005	
2	0·6330	0·6327	0·0003	0·00046
3	0·6550	0·6544	0·0006	

A second test was carried out, with the filter paper arranged on the surface of beads, to correspond as closely as possible to the arrangement to be adopted for the inoculation experiments (see Fig. 1). The cellulose was recovered from the flask by first decanting as much of the liquid as possible; Schweitzer's reagent was then poured in, and, when the paper had dissolved, the beads were washed free of the reagent, both the reagent and the washing being added to the dilute HCl. The amounts of cellulose that were recovered are here given.

Weights in grammes.

No.	Filter paper (at start)	Cellulose recovered	Loss	Average
1	1·3410	1·3300	0·0110	
2	1·3370	1·3280	0·0090	0·0100
3	1·3450	1·3350	0·0100	

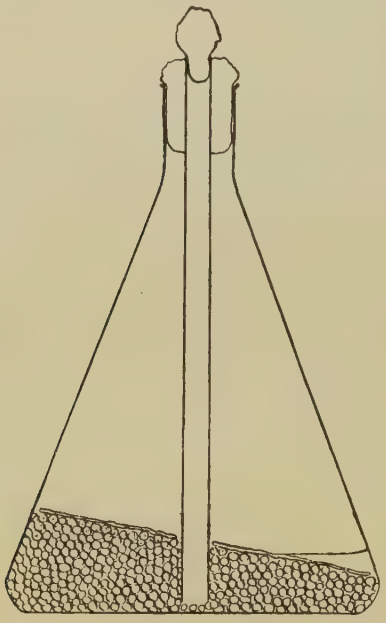


Fig. 1. Showing the orientation of the filter paper on the surface of glass beads, and the tube for inoculation.

Experiment I.

For the first experiment 500 c.c. Erlenmeyer flasks were filled to a depth of about 2 cms. with small glass beads which had been washed with HCl and then with hot water. Upon the surface of these was laid a weighed sheet of filter paper, which was folded in such a manner as to lie flat on the beads. Through the centre of this paper passed a glass tube reaching to the floor of the flask, the upper end, plugged with cotton wool, passing through the plug in the neck of the flask (Fig. 1). A solution consisting of 50 c.c. of the mineral salts solution and 50 c.c. of distilled water containing 0·1 per cent.[1] of the compound to be tested, was run into the flask *via* the glass tube. Control flasks were set up in which 50 c.c. mineral salts solution and 50 c.c. of distilled water alone were added to the beads and filter paper. Sterilisation was carried out in the autoclave at 15 lbs. pressure for 15 minutes. Each flask was inoculated *via* the tube with 1 c.c. of a suspension of a 4-day old culture

[1] For the reason stated in the text the concentration of lignin was much less than this.

of *M. agar-liquefaciens* and incubated for 10 days at 22° C. Duplicates were set up, using sand instead of beads, but this method was found to give conditions of insufficient aeration, and these cultures were not analysed in consequence.

After three or four days incubation the flasks were examined for signs of decomposition. A slight yellowing of the paper was evident, and it was becoming soft and pulpy. On gently shaking, the liquid was seen to be full of fine strands of filter paper. By the 10th day, when the whole of the paper had apparently broken down into these small fibrils, estimations of the amounts of cellulose remaining were carried out. The results of this experiment are given in Table I.

Table I.

Additional substance	Weight of paper at start (gm.)	Weight of paper recovered after 10 days (gm.)	Cellulose lost per cent.
Dextrin	0·607	0·370	39·04
Lignin	0·545	0·337	38·165
Xylose	0·580	0·360	37·93
Sucrose	0·555	0·380	31·54
Arabinose	0·585	0·426	27·18
Straw gum	0·585	0·465	20·51
Sol. starch	0·595	0·517	13·11
Control	0·587	0·548	6·64
Glucose	0·565	0·533	5·66
Mannitol	0·615	0·605	—

The amount of additional substance in each case was 0·05 per cent. of the liquid medium, save in the case of lignin. A small part only of the lignin went into solution. This substance and the straw gum were autoclaved previously for 20 minutes at 20 lbs. pressure. The straw gum went completely into solution, but only 18 mgm. of lignin dissolved in 100 c.c. H_2O. This concentration was then halved in making up the medium, giving 9 mgm. of lignin in the 100 c.c. used.

The control culture in this experiment did not show such good growth as usual because the slope of the beads was displaced and was so steep as to exclude air from a large surface of the paper. This experiment was regarded, however, merely as a preliminary one to ascertain which of the compounds used would be worthy of a more critical test.

Experiment II.

It was decided to continue with xylose, arabinose, lignin, and straw gum in a second experiment. Each substance was tested in two concentrations, 0·025 and 0·05 per cent., and duplicate flasks of each con-

centration were set up, the filter paper resting on the surface of glass beads as described in the first experiment, with the exception that the surface of the beads was kept horizontal. Duplicate controls, inoculated, but with no organic compound other than the cellulose, were also set up. The cultures were incubated at 22° C. for 10 days, and the amount of cellulose remaining unattacked at that time determined as described above. The results of this second experiment are shown in Table II.

Table II.

Substance added	Weight in gm. in solution	Paper at start		Paper recovered		Cellulose lost per cent.		Average loss per cent. of duplicates
		A	*B*	*A*	*B*	*A*	*B*	
Xylose	0·025	0·777	0·858	0·466	0·498	40·0	41·9	40·95
	0·05	0·737	0·715	0·435	0·429	40·9	40·0	40·45
Lignin	0·0045	0·895	0·717	0·541	0·431	39·55	39·9	39·73
	0·009	0·772	0·853	0·501	0·562	35·1	34·1	34·60
Arabinose	0·025	0·808	0·783	0·566	0·522	30·0	33·3	31·65
	0·05	0·807	0·793	0·597	0·588	26·0	25·8	25·91
Control	—	0·868	0·795	0·625	0·517	27·99	34·97	31·5
Straw gum	0·025	0·885	0·800	0·730	0·656	17·5	18·0	17·75
	0·05	0·885	0·770	0·708	0·624	20·0	18·96	19·50

It will be seen from the above table that xylose and lignin produce a very considerable increase in the amount of cellulose which is broken down in 10 days by this organism. Arabinose had no stimulating effect while straw gum caused a depression of its activity. These figures are consistent with those of the previous experiment, except in the case of the control which in the first experiment was abnormal but on this occasion gave normal growth.

The stimulating effect of xylose and lignin is of especial interest in view of the occurrence of the pentose groups in straw and of the association of lignin with cellulose in vegetable tissues. In considering the possible cause of this stimulation it was thought that it might be important that in the case of lignin the smaller concentration alone gave a significant stimulation, and in the case of arabinose the higher concentration had a depressing action. The optimum amount of such a stimulating substance may be very small, a larger quantity merely reducing the amount of cellulose destroyed by supplying an alternative source of energy. In order to ascertain whether the organism were deriving energy from the added substance the following experiment was set up.

Experiment III.

Test tube cultures were made containing 10 c.c. of the mineral salts solution, with the same concentrations of xylose, lignin, arabinose and straw gum as in the last experiment. Four tubes of each concentration contained a strip of filter paper, and four were without. Inoculations were made with a loopful of a thick suspension from the liquefied portion of an agar slope in physiological salt solution, and the cultures incubated at 25° C. Examinations were made from day to day and the following results were noted.

With filter paper.

1.	Duplicate controls		Paper eaten through on the 5th day.
2.	Lignin	0·0045	Same as controls.
		0·009	Paper eaten through in two tubes on the 6th day, and one on the 7th. One failed.
3.	Straw gum	0·025	Paper eaten through in two tubes on the 5th day, one on the 7th, one on the 9th.
		0·05	Paper eaten through in one tube on the 5th day, one on the 6th, and two on the 9th.
4.	Arabinose	0·025	Paper eaten through in one tube on the 9th day, and one on the 14th.
		0·05	Paper not attacked.
5.	Xylose	0·025	Paper eaten through in one tube on the 9th day.
		0·05	Paper not attacked.

Without filter paper.

The organism grew well on straw gum, especially in the higher concentration, in 3 days. None of the other substances supported any growth.

In test tube cultures, then, none of these compounds stimulate the organism to greater activity in destroying cellulose, but, on the contrary, are inclined to retard it. Straw gum alone of these four compounds supplies it with an alternative source of energy.

Experiment IV.

In considering the stimulating action of xylose on the decomposition of cellulose it was important to ascertain whether this was due to a hastening of the initial growth of the organism and to a consequent shortening of the time before it commenced to affect the cellulose. To test this point a series of test tube cultures was set up with a wide range of concentrations of xylose, and the time taken for a visible attack of the cellulose was noted. Four tubes of each concentration were set up, the mineral salts being of the same concentration in each series, and

inoculated with a drop of a thick suspension from a small separating funnel. The concentrations of xylose were in the percentages as under:

1·0	0·5	0·25
0·1	0·05	0·025
0·01	0·005	0·0025

Four tubes with no xylose were incubated as controls. Incubation was carried out at 25° C., and examinations made daily. The times were noted at which the paper was (I) showing the first signs of being attacked,

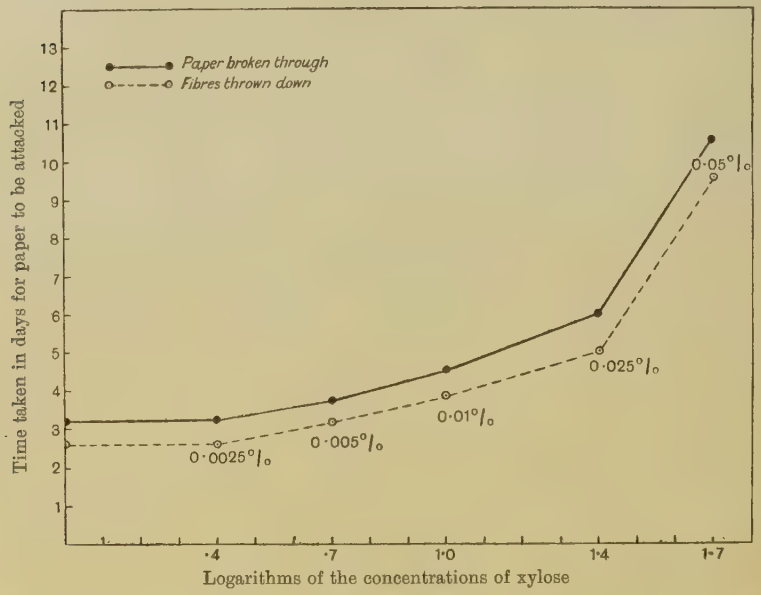


Fig. 2. The effect of varying concentrations of xylose in delaying the action on filter paper by *M. agar-liquefaciens*.

which was indicated by the throwing down of fibres into the liquid, and (II) eaten through; this latter was easily seen if the tubes were shaken gently. The appended graph shows the concentrations of the added xylose plotted against the time at which the first signs of attack and the breaking of the paper occurred (Fig. 2).

Concentrations above 0·05 per cent. are not included in the above graph, as no growth occurred in any of them. The points plotted represent the mean of four tubes, except in the case of 0·05 per cent. where only two tubes showed the required characteristic. The figures ·4 to 1·7

are the logs. of the milligrammes of xylose per 100 c.c. of culture fluid. In the control cultures the paper was broken through in the same time as in the case of the lowest concentration.

The curve obtained from this experiment shows that in test tube cultures concentration of xylose above 0·25 mg. in 10 c.c. extends the time required for the paper to be broken through at the air-liquid level, and that at no concentration tested did the xylose shorten this period. This delaying action of xylose in all but the weakest concentration is apparently not due to the carbohydrate acting as an alternative source of energy since the delaying concentration is so small that the energy supplied by it seems negligible. Moreover, Exp. III indicates that the organisms do not use xylose or lignin as a source of energy.

With regard to the mode of action of small amounts of xylose and lignin, in increasing the amount of cellulose decomposed, the view that these substances supply enough energy to hasten the initial growth of the organisms is rendered improbable by the considerations mentioned above, and also by the fact that, at any rate in conditions of test tube cultures, the weakest concentrations of xylose tested did not shorten the period taken by the organism to commence attacking the cellulose.

The fact that xylose and lignin may be stimulating or harmful to cellulose decomposition according to their concentration seems to be of great interest in view of the varying ratios of cellulose, lignin and pentosans met with in natural vegetable products. It seems possible that a continuation of this line of investigation may help to explain the varying rates at which different classes of plant residues are decomposed.

Summary.

(1) A new micro-organism is described that possesses the power of decomposing cellulose (filter-paper) and agar-agar with great rapidity. It is able to utilise either of these substances as the sole source of energy for its growth. No loss of the power to decompose cellulose or agar has been observed after nine months cultivation in the laboratory.

(2) On account of its morphology and physiological characters the organism has received the name *Microspira agar-liquefaciens*.

(3) Pure culture experiments with this organism show that under conditions of adequate aeration it will decompose filter paper to a greater extent in the presence of small quantities of certain other carbohydrates and related substances. The stimulating effect of xylose and lignin is of especial interest owing to their association with cellulose in straw.

We are much indebted to Mr H. G. Thornton for his valued help and criticism during this work; to Mr S. du Toit for supplies of xylose and lignin, and for the estimation of lignin in solution; and to Mr G. C. Sawyer for estimations of the amount of nitrogen in filter paper and agar.

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EXPLANATION OF PLATES XII AND XIII.

PLATE XII.

Cultures of Microspira agar-liquefaciens.

Fig. 1. Test tube culture three days old showing filter paper broken through. (Nat. size.)
Fig. 2. Nutrient agar slopes. On the left a sterile slope; on the right a streak culture of *M. agar-liquefaciens* showing increase in liquid at the bottom. (Nat. size.)
Fig. 3. Five days old agar plate showing depressed colonies and refraction of light from semi-liquefied agar. ($\times \frac{2}{3}$.)

PLATE XIII.

Camera lucida drawings of M. agar-liquefaciens.

Fig. 1. *M. agar-liquefaciens* showing flagella. Stained by P. H. H. Gray's (Rothamsted) method. ($\times 2500$.)
Fig. 2. Fibre of filter paper (three days old culture) showing organisms inside the central canal. Unstained. ($\times 1200$.)
Fig. 3. Fibre of filter paper, one month old, showing organisms in the coccoid stage inside the central canal. Stained *intra vitam* with carbol fuchsin. ($\times 1200$.)

(*Received March 26th*, 1924.)

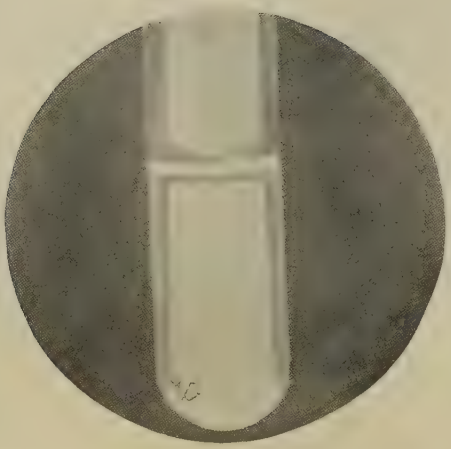


Fig. 1.

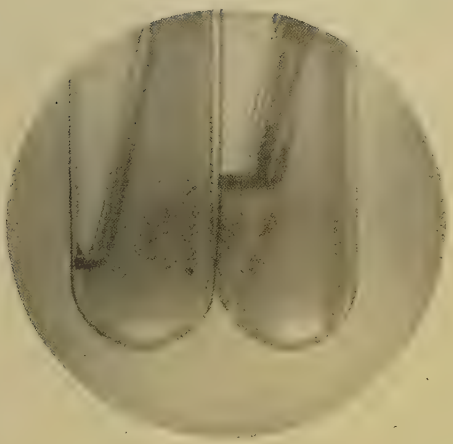


Fig. 2.

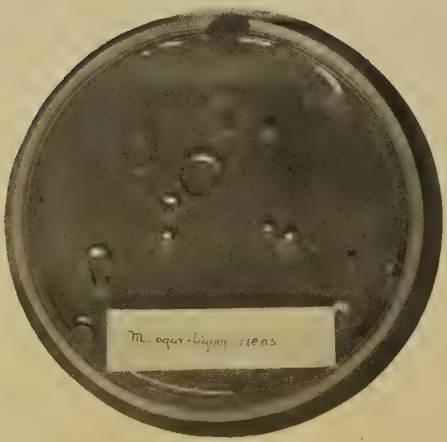


Fig. 3.

GRAY & CHALMERS.—On the Stimulating Action of certain Organic Compounds. (pp. 324 –338.)

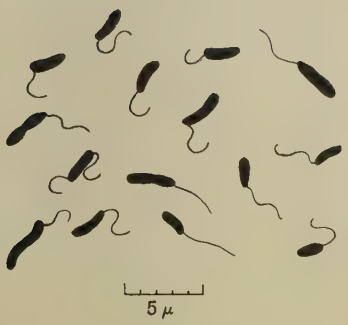


Fig. 1.

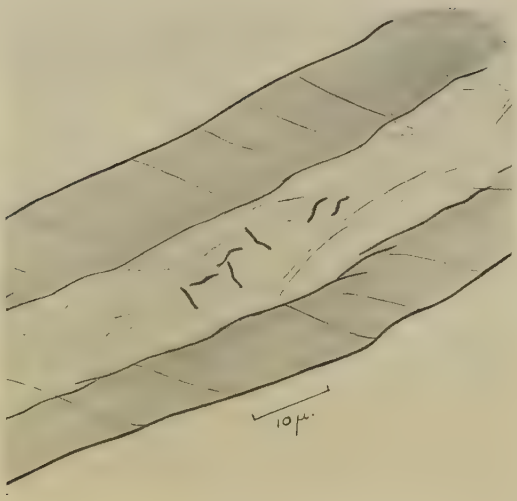


Fig. 2.

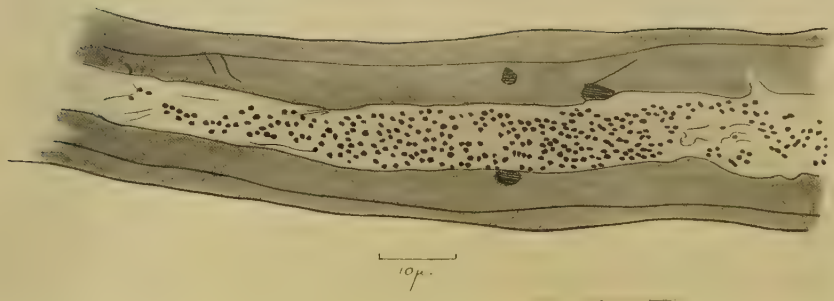


Fig. 3.

GRAY & CHALMERS.—On the Stimulating Action of certain Organic Compounds. (pp. 324—338.)

OBSERVATIONS ON THE LIFE HISTORY OF TAPEWORMS OF THE GENUS *MONIEZIA*

By J. R. W. JENKINS, B.Sc.
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(With Plate XIV.)

It is a remarkable fact, that whilst numerous species of tapeworms belonging to the genera *Moniezia* (Blanchard, 1891); *Thysanosoma* (Diesing 1834); and *Stilesia* (Railliet 1893), are common parasites of herbivorous animals, in no case has the intermediate host of the parasite been discovered.

The present paper records an attempt to elucidate the life histories of those species of *Moniezia* commonly parasitic upon lambs in the Aberystwyth district, and although the conclusions reached are largely negative, it is put forward in the hope that some portion of the ground may be cleared for other investigators.

Numerous investigators have embarked upon the problem, the results of their observations having recently been dealt with by Flattely(1), who gives a most comprehensive historical account of their work, together with an account of his own investigations on the subject, and a full bibliography, to which the reader is referred.

Although some authorities support the theory of direct infection without the intervention of an intermediate host, numerous attempts to substantiate the theory have been fruitless, and the observations here described were made on the assumption that the life histories of *Moniezia* spp. are in accordance with those of the great majority of Cestodes—presupposing, therefore, the existence of an intermediate host.

The outstanding feature of the infection of lambs with *Moniezia* spp. is the very early age at which infection occurs. On March 17th, 1922, at a farm in the Aberystwyth district, mature proglottides were found in lamb droppings, just over five weeks from the commencement of the lambing season. Since a lamb of five weeks old is not weaned, and does not nibble pasture in more than a very casual manner, the fact that heavy infections occur at such an early age has led many investigators

to support the theory that infection takes place from the mother, either *via* the maternal milk, or from external parasites living on the surface of the udder, and taken in by the lamb during sucking operations, or from some larval stage of the tapeworm which, present in the herbage, becomes transferred to the udder when the mother ewe lies down. In the absence of evidence to the contrary, the theory that infection is contracted from the mother ewes receives support from Flattely(1); it is considered, however, that the observations here recorded render the theory untenable.

Period of the year during which infection occurs.

The majority of infections take place in the spring, when the lambs are a few weeks old, and it has been hitherto supposed that infection is confined to that period. It has now been established, however, that infection is not confined to the spring. On October 12th, 1921, fifteen tapeworms were collected from lambs which had been born the previous spring. Seven of these tapeworms were mature, varying in length from 94 cm. to 138 cm.; they had evidently shed many proglottides, and were the outcome of an infection which had taken place the previous spring. The remaining eight tapeworms were complete, that is, the original end segments possessing the terminal excretory pore were present, showing that no ripe segments had been shed. The most posterior segments, although possessing well-developed uteri, were by no means fully mature. These worms were evidently the result of an infection which took place at a much later date than the one which gave rise to the seven worms referred to above. Since a tapeworm collected on March 31st, 1922, from a lamb four months old, whilst possessing the terminal segment with excretory pore, was more fully mature than these eight, it is probable that the infection to which the latter were due took place at least subsequent to July, 1921.

Duration of the period of infection.

Since it is not possible to examine lambs in any number, for tapeworm infection, until they are slaughtered, usually at about four months old, and since the rate of growth of the tapeworms is not known, it is not possible to fix accurately the earliest date at which infection may occur. As stated above, however, mature proglottides were being shed on March 17th, 1922, and since the longest worm complete with terminal segment which has been collected measured 240 cm., it is clear that for

mature proglottides to be shed on March 17th, infection must have taken place some little time prior to that date.

On May 18th, 1922, two hundred and thirty tapeworms were taken from a single lamb aged about four months. Care was taken when enumerating them, to count only those possessing scolices. Eight of these tapeworms were broken, the remaining two hundred and twenty-two were unbroken, and had shed no segments, each possessing the original terminal segment. These tapeworms varied in length from 123 cm. to 3 mm., the variation being gradual as shown by the following table. Measurements for the sake of briefness have been reduced to the nearest ·5 cm.

Length in cms.	No.	Length in cms.	No.
123	1	11	3
106	1	10·5	2
38	1	9·5	1
30·5	1	8	7
28·5	1	7·5	6
27	1	7	3
26·5	1	6	11
25·5	2	5·5	2
24·5	1	5	11
24	1	4·5	5
23·5	1	4	12
20	2	3·5	12
19	1	3	22
17·5	1	2·5	21
16	3	—	—
15·5	1	2	20
15	1	1·5	23
14·5	1	—	—
14	1	1	22
12·5	2	·5	12
12	2	—	—

Assuming all the tapeworms to have grown at the same rate, it would appear that infection had been continuous for some weeks up to the middle of May, and that the later infections were the heaviest.

The fact that all the tapeworms resulting from larval forms ingested do not necessarily persist has not been lost sight of, and is dealt with below. This table should therefore be read in conjunction with the discussion as to the probable period of maximum infection, which follows.

It was found impossible to obtain reliable statistical information as to the exact period of maximum infection, for the following reasons:

A. It was not possible to examine the intestines of every lamb slaughtered.

B. During the period when the lambs slaughtered were found to be most heavily infected, the large number of tapeworms occurring in each lamb (230 being the greatest number found in any one lamb) made it impossible to count and measure all the tapeworms collected, before post-mortem decay set in. All disentanglement of worms—no light undertaking—had to be done before fixing, as the worms after fixing were too brittle to permit much handling. All measurements had also to be made before fixing, as considerable expansion or contraction may occur during the fixing process, making post-fixation measurements not strictly comparable one with the other.

Reliable figures indicating the intensity of infection at different dates are thus not available, but it can be stated with confidence that general observations show infection to commence about the beginning of March (see above), that the rate of infection increases until the end of April or the beginning of May, when it is at its maximum. The rate decreases after that date, and infection may occur at least until the beginning of August (see above).

These conclusions are arrived at by observations, not of the periods when most tapeworms were found in the lambs, but of the periods when most young tapeworms indicating the occurrence of a recent infection were found. This procedure was necessary owing to the fact that the older the lambs get, the smaller the number of tapeworms they are found to harbour. Two possibilities suggest themselves in explanation of this fact, firstly, that it is probably a physical impossibility for a lamb to harbour a large number (say 200) of fully grown worms, and that some are forced out of the intestine by the growth of those more securely attached to the intestinal wall; and secondly, that the probable change in the digestive juices, consequent to the change from a milk diet to a pasture diet, creates an environment less favourable both to the development of any larval forms which may be ingested, and to the continued presence of those tapeworms already harboured.

Liability of sheep to infection.

It has hitherto been supposed that lambs only are liable to infection by *Moniezia* spp., and that any tapeworms found in sheep have persisted from an infection which occurred when the sheep were lambs.

On March 16th, 1922, a small *M. translucida* was collected from a three year old sheep. Although the scolex was unfortunately not present, from the fact that the most anterior segments showed no trace of the rudiments of reproductive organs, and the original terminal segment,

complete with excretory pore, was present, it was clear that the whole tapeworm could not have exceeded 10 cm. in length. It was, therefore, the result of a very recent infection.

On April 4th, 1922, a young tapeworm, 7 mm. long, was taken from a yearling sheep born the previous spring. The worm showed no trace of segmentation and was obviously the outcome of a very recent infection.

These observations have since been confirmed by other workers at Aberystwyth, who have also taken young tapeworms from yearlings and from adult sheep. It seems probable that infection both of sheep and of lambs may occur at any period of the year, but that the digestive juices consequent to a herbivorous diet are, as mentioned above, not favourable to development of the larval form.

The observations recorded above show that infection is not confined to the sucking period, and may take place long after the animal concerned has been weaned. They appear, therefore, to render the following theories untenable:

A. *That infection occurs* via *the maternal milk, or from larval forms existing externally upon the maternal udder.*

B. *That a milk diet is essential to development of the larval form.* The fact that lambs are infected to an enormously greater extent than are sheep, has given rise to the theory that a milk diet is essential for the development of the larval stage. These observations show that a milk diet, while it may be favourable to such development, is not essential.

Distribution of the genus.

In ascertaining the distribution of *Moniezia*, farms have been regarded as infected, only when tapeworms have been collected from lambs bred on those farms, and reared there up to the time of slaughtering. Owing to the fact that lowland farmers are in the habit of buying in sheep from the uplands in the Autumn, the presence of *Moniezia* in a sheep is not necessarily an indication that the farm from which it was sold for slaughtering is infected.

In all, thirty-four farms were found to be infected. It was hoped that some correlation might be established between the physical characteristics of a farm and the presence or absence of *Moniezia* infection, and thus, that a clue would be obtained to the life history of the parasite. Unfortunately observations have not revealed the existence of any such correlation; farms of all types were found to be infected, and no factor common to all, and giving a clue to the life history, could be discovered.

Infected farms were found from sea level to 600 feet; wet marshy

farms, dry hillside farms; farms on light land and farms on heavy land, were all infected, whilst the characteristic vegetation varied with altitude, type of soil and moisture content. One instance in particular may be mentioned. A certain farm near Aberystwyth is divided into two clearly defined portions, one consisting of dry hillside pasture land, running up to about 200 feet, the other of low lying water-logged land, merging into a marsh, practically at sea level. The latter low lying portion was sublet by the farmer, and on it the sub-tenant raised a crop of lambs. On the dry hillside pastures the farmer also raised a crop of lambs. At no time did the two flocks intermingle, but were kept entirely separate. Lambs from both flocks were found to be equally heavily infected with *Moniezia*.

As far as present observations show, therefore, altitude to 600 feet, type of soil, water content of soil or type of vegetation, do not appear to be factors influencing the infection of lambs with *Moniezia*.

Fauna of infected farms.

An examination of the fauna of infected farms during the infective period showed that the variety of possible intermediate hosts present on pastures at, and immediately after, lambing time, when infection starts, is very small, and is practically limited to field slugs, dung beetles and dung-living Staphylinids and Mites, all clearly living under the best possible conditions for the ingestion of tapeworm eggs.

Since, as described above, it is possible for a lamb to harbour very large numbers of tapeworms, three possibilities must be considered:

A. The intermediate host may be extremely common on the pastures at lambing time.

B. The intermediate host may not be common, but may harbour very numerous tapeworm larvae.

C. The intermediate host may neither be common, nor harbour large numbers of larvae, but the larval form may be of a multiple type, *i.e.* either a Coenurus or an Echinococcus.

It is, of course, possible for all three conditions to be co-existent, but further consideration shows that the first at all events must be held to exist. If this were not so, although individual lambs might be found to be heavily infected by the existence of conditions two and (or) three, one would expect infection to be patchy, that is, that a small proportion of lambs only in any flock would be found to be heavily infected, and that the remaining lambs would not be infected at all. This, however, is not so; when a flock is infected with *Moniezia*, practically all the lambs

are infected, and the degree of infection of individual lambs varies from very heavy to very light.

One is thus forced to the conclusion that the intermediate host is very common, and this conclusion has importance in that, excluding the external parasites of sheep and lambs, it narrows down the possible intermediate hosts to members of the groups detailed above.

With regard to the possible occurrence of condition "B," that is, that the intermediate host may harbour large numbers of the larval forms, the supposition that this is so has its attractions. It would help to explain, for instance, why the percentage of lambs infected is very high, having regard to the fact that at the time of maximum infection, the lambs do very little nibbling of pasture. Thus, if the intermediate hosts were common, and also harboured large numbers of larvae, a lamb, although little nibbling were done, might easily ingest a single intermediate host, and so become heavily infected at once. It must be remembered, however, that where a lamb is heavily infected, the worms are not found to be all of the same age, as one would expect, if infection occurred through the ingestion of a single intermediate host. On the contrary, the worms are all of different ages, as shown by the figures tabulated above, indicating, therefore, the ingestion of numerous intermediate hosts.

The existence of the third condition, however, does not seem probable. It seems extremely likely that the intermediate host, if one exists, will prove to be some small invertebrate, and that the larval form will follow the usual rule in such cases, and will be of the Plerocercoid or Cysticercoid type. Having then been forced to the conclusion that the intermediate host must be extremely common, attempts were made to produce infection of possible hosts by feeding them with mature proglottides. Field slugs and dung beetles being the commonest of the pasture living forms at lambing time, attention was confined to these two groups, and in the Spring and Summer of 1922, the following experiments were carried out.

As a preliminary to the feeding experiments, the whole intestinal contents of two lambs, from each of which very young tapeworms of from 2 mm. to 5 mm. in length had been taken, were examined in the hope that since infection had taken place very shortly before, some recognisable relics of the intermediate hosts might be discovered. The whole of the intestinal tract was washed out with water, the washings sedimented several times in glass cylinders, and the whole of the solid residue carefully examined. The result unfortunately was entirely negative.

Feeding experiments.

With Arion ater. Six slugs were fed for five days on ripe proglottides, and afterwards killed, one per day, and examined.

With Agriolimax agrestis. Two batches of slugs, one of fifteen, and the other of seventeen, were fed with mature proglottides for five days, and were then killed and examined at the rate of three per day.

Both species of slugs devoured the proglottides voraciously.

Two methods of examination were adopted:

A. *By dissection.* The slugs were carefully dissected under the binocular microscope, and whilst the whole of the animal was examined, particular attention was paid to the pulmonary and body cavities, and to the digestive system, the contents of the alimentary canal being removed and examined separately.

B. *By serial sections.* One specimen of *A. ater* and three of *A. agrestis* were stained with borax carmine, and examined by means of serial sections.

By neither method of examination was the slightest evidence of any development of the tapeworm eggs obtained. An examination of the faeces of the slugs showed that the majority of the eggs passed through the alimentary canal unchanged, whilst a few showed traces of what were apparently disintegration changes.

With dung beetles. The following species of dung beetles are all common in sheep dung in the Aberystwyth district, and were subjected to feeding experiments:

Onthophagus nuchicornis	5	specimens fed
Aphodius fossor	16	,,
A. fimetarius	11	,,
A. merdarius	9	,,
A. haemorrhoidalis	19	,,

The beetles were fed with mature proglottides, which they eat with avidity, for from three to five days and were killed and examined at daily intervals up to three weeks. Examination was by means of dissection under the binocular microscope, whilst the contents of the alimentary canal were removed and examined separately. Results were entirely negative.

Experiments with sheep keds.

The Sheep Ked, *Melophagus ovinus*, was next considered as a possible intermediate host. Since sheep must often lie down on dung containing mature proglottides, it is extremely likely that the wool at times must

harbour large numbers of *Moniezia* eggs, and therefore that the Ked almost certainly must often ingest them. Keds were therefore collected at the slaughterhouse from the fleeces of lambs found to be infected with tapeworms, and were carefully examined, by dissection. Thirty-two Keds were thus dealt with, with entirely negative results. Attempts to keep Keds alive in captivity, and to feed them with *Moniezia* eggs, failed.

In all feeding experiments care was taken to provide mature proglottides from as many species as possible.

Details of species collected.

The following five species of *Moniezia* have been collected at the slaughter house, Aberystwyth.

Alba Group. M. alba (Perroncito, 1879).
Planissima Group. M. benedeni (Moniez, 1879).
M. pellucida Blei, 1921
M. translucida Jenkins, 1923.
Expansa Group. M. expansa (Rudolphi, 1810).

In addition, three other forms have been collected, whose specific position has not been determined. *M. trigonophora* (Stiles and Hassall, 1893), taken by Flattely at Aberystwyth(1), has not been taken by the writer.

It should be added that Flattely does not wish to attach too much importance to the occurrence of *M. trigonophora* in Wales, on account of the fact that he has not found it elsewhere, and that the triangular arrangement of the testicles, one of the diagnostic features, is occasionally shown by *M. expansa.*

A study of the genus *Moniezia* convinces one that a revision of the genus is extremely desirable. As has already been pointed out by the writer(2), forms are at present given specific designation on descriptions far too scanty to justify their inclusion in the genus even, the descriptions often being confined to the most meagre details of externals. Identifications based on observations of externals are useless unless they are supplemented by details of the internal anatomy; not only may two members of the same species differ so much externally as to be mistaken for different species, but two members of different species may resemble each other so much as to be mistaken for members of the same species.

The enormous number of tapeworms occurring during the period of maximum infection, and the fact that identification must be based on both external and internal anatomy, makes it impossible to be sure that

all species occurring in the district have been identified and also to obtain reliable statistics of the frequency of occurrence of each species. The writer is of the opinion that further observations would reveal the occurrence of other species in addition to the ones already identified.

With regard to the number of species occurring in any one individual, the usual condition is that the tapeworms found in one lamb are of one species. The rule, however, is not invariable, as *M. expansa* and *M. translucida* have been found in one individual, as have *M. expansa* and *M. alba*.

SUMMARY.

The period during which lambs may become infected with *Moniezia* spp. is not confined to the Spring, but may extend at least until August.

Sheep as well as lambs may become infected.

Neither the distribution of the genus nor the fauna of infected farms has been of guidance in discovering the life history.

Examination of the intestinal contents of lambs which had become heavily infected shortly before death gave negative results.

Attempts to produce infection of possible intermediate hosts by feeding with mature proglottides gave negative results.

Five species of *Moniezia* have been collected at Aberystwyth, and there is every reason to suspect the occurrence of further species.

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(2) JENKINS, J. R. W. On a New Species of *Moniezia* from the Sheep, *Ovis aries*. *Annals of Applied Biology*, X, No. 2, July, 1923.

EXPLANATION OF PLATE XIV.

Fig. 1. Young tapeworm collected on April 4th, 1922, from a yearling sheep born the previous spring. Segmentation has not commenced.

Fig. 2. Terminal segments of a young specimen of *M. translucida* collected on March 16th, 1922, from a three year old sheep. The genital organs are still in *anlage*, and the terminal excretory pore is present.

Fig. 3. Terminal segments of a young specimen of *M. translucida* collected on March 31st, 1922, from a lamb four months old. This specimen is clearly much more mature than that illustrated by Fig. 2. Fertilisation has been effected as shown by the swollen receptacula, whilst the uteri have spread considerably.

(*Received April 3rd*, 1924.)



Fig. 1.



Fig. 2.

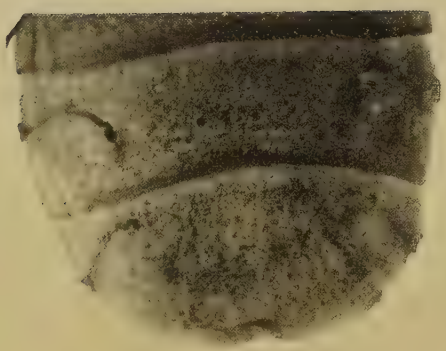


Fig. 3.

JENKINS.—Observations on the Life History of Tapeworms of the Genus *Moniezia*. (pp. 339—348.)

THE SOIL POPULATION. AN INVESTIGATION OF THE BIOLOGY OF THE SOIL IN CERTAIN DISTRICTS OF ABERYSTWYTH

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(With 7 Text-figures.)

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1. INTRODUCTION.

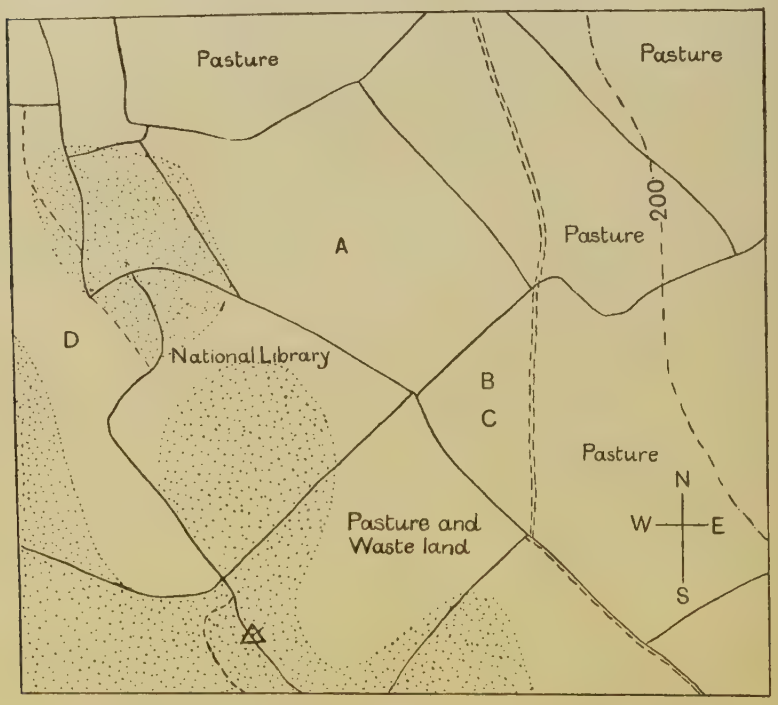
The following paper gives an account of a systematic investigation of the soil from certain areas at Aberystwyth, extending over a period of more than two years.

The investigation is mainly concerned with a quantitative seasonal examination of the soil fauna, together with some reference to the vegetation, humus content, physical nature of the soil and to the atmospheric conditions. An attempt has been made to include all organisms in the soil which are visible to the naked eye or by using a hand lens. The observations recorded offer an interesting comparison with those obtained by Cameron (4, 5), Morris (19, 20) and Buckle (3).

A preliminary study of the soil fungi has been carried out, the results of which, however, are not included in this paper, except in so far as they are directly concerned with the soil fauna.

The work has been carried out on land belonging to the College plant breeding station, just north of Aberystwyth, about two-thirds of the distance up the valley side of the Rheidol river, and about three-quarters of a mile from the coast.

The particular areas examined are within a short distance of each other, and were chosen as appearing to be typical of their kind in the



A. Pasture field. B. Ungrazed grassland. C. Land recently ploughed.
D. Cultivated land. ⁞⁞⁞ Terannon, Llandovery.

Fig. 1.

district. They agree in that they are formed of soil essentially the same in physical, chemical and geological character. They consist of: (*a*) PERMANENT PASTURE which forms the main topic of the paper; (*b*) GRASSLAND similar in nature to the pasture, save that it is ungrazed; (*c*) land from which the turf had been entirely removed a short time before the investigation and (*d*) land under cultivation.

The land of the Aberystwyth district consists mainly of shales and

grits from the Ordovician and Silurian systems, overlain in many parts by boulder clay or alluvial deposits. The upper layers of the areas investigated consist of the Terannon formation of the Llandovery (Silurian) overlain by boulder clay (Fig. 1), so that the soil is probably formed by an intermingling of these. The majority of stones present in the soils are either of the boulder clay type or fragments of laminae from the shales. The soil is fertile with a moderate amount of humus to a depth of at least nine inches and shows a good growth of vegetation.

The physical characters of the soil allow free gaseous diffusion among the particles, thus affording favourable conditions for life within the soil. Aeration is further increased by the long and spreading tap root systems of certain plants which aid in opening up the soil. Hole(13) in his paper on "Soil Aeration" states that in ordinary arable or pasture land, the air differs little in its composition from atmospheric air, the amount of oxygen being somewhat less and the carbon-dioxide a little more. It is probable that the degree of aeration of the soil is one of the main factors regulating the vertical distribution of the macroscopic fauna; aeration decreases with depth. When the soil is loosened by ploughing or hoeing aeration is efficient for a greater depth with a correspondingly deeper distribution of the fauna. The family *Achorutidae* of the *Collembola* illustrate this particularly well, the numbers obtained from the 6 to 9 inch layer almost equalling those from the 3 to 6 inch or the surface 3 inch layer. This is in marked contrast with grasslands, where, in the investigation, macroscopic organisms were to a marked degree most numerous in the surface 3 inches, the numbers becoming steadily reduced in the lower layers, specimens occurring but rarely below 12 inches.

Again, a number of soil organisms are root feeders and would thus, particularly in grassland, be concentrated in the upper layers of soil.

Another main factor influencing vertical distribution is the occurrence of humus.

Ploughing and hoeing have a destructive effect on many forms, and this fact was most clearly demonstrated by records of certain samples where the numbers of the *Collembola* reached 169 in a sample from an undisturbed portion of arable land, against 25 from a part hoed a few days before examination. Apart from direct injury to larvae and the larger forms, the disturbance of the soil naturally results in increased evaporation of moisture from the surface, causing temporary drought. As stated by certain investigators, this has a particularly detrimental effect on *Collembola*.

Excessive ground water appears to be the greatest mechanical enemy

of the soil fauna, but in the areas investigated there is little danger of water-logging owing to the geological formation of the land and the physical nature of the soil.

The average and equable conditions to which the areas are subjected are indicated by the typically mesophytic vegetation.

Fungal attack has been small, and, so far as observed, restricted to injured organisms. Also, there has not been any excessive occurrence of the recognised harmful types of fauna; such were present but were only found occasionally, either singly or in small numbers.

2. METHOD FOR INVESTIGATION OF THE SOIL FAUNA.

(*a*) Collecting. Samples of a 9-inch cube in size were taken and were collected in three layers, each $9 \times 9 \times 3$ inches, to obtain a quantitative estimation of the soil fauna at different depths. Samples were taken several feet apart from each other.

(*b*) Examination of the samples. After experiment, the best method of procedure has been found to be as follows:

(i) A preliminary sorting of the soil particles over brown paper and examination for organisms.

(ii) After this preliminary sifting, a small portion of the soil is again taken and placed in a sieve of about ·75 mm. mesh and a slow stream of water allowed to run in. The sieve is at the same time shaken, to allow the fine silt to pass through the mesh, and this is caught in a wide shallow dish. When no more sediment passes through both residue and filtrate are examined.

Although examined time after time with a high power hand lens, no organism has ever been found in the filtrate, so it may be accepted that no macroscopic forms pass through the sieve. The smallest organisms taken into consideration in this investigation appear as tiny specks to the naked eye. After the preliminary experiments the filtrate was neglected.

The residue is carefully washed out from the sieve into a wide shallow dish and water added until the dish is half full; the residue is stirred gently, thus releasing any organisms between the particles, which then float up to the surface of the water. The latter is examined with a hand lens or a binocular microscope. Several organisms which are difficult to detect by the first sorting are revealed by this method.

The water is poured off and the process repeated with fresh water until nothing further is found; usually once is sufficient.

Finally, the actual solid residue is examined, though at this stage there is rarely anything present.

It is necessary to go through the preliminary sorting, as the larger forms are very liable to be bruised during sieving, though the smaller ones escape uninjured. The larger pupae especially were in some cases so damaged that it was impossible to rear them for purposes of identification.

3. PERMANENT PASTURE LAND.

(i) ECOLOGY. The chief area to be discussed is a field of permanent pasture, about 2060 square feet in area, and approximately 200 feet above sea level. It is surrounded by other pasture fields to the S.E., E. and N., one of which was an ungrazed area for several years, but since January 1921 has been used for agricultural experiments with wheat and grasses. A strip of waste land lies between the area in question and the buildings of the National Library.

Two sides of the pasture field are bounded by thin hawthorn and blackthorn hedges, on vertical banks about 4 feet in height. These hedges are clean with little variety in vegetation beneath them. The remaining sides of the field are marked by wire.

A stream runs across an adjoining field, but apparently has little effect on the fauna of the neighbouring land.

The field has a fairly uniform surface, evenly covered by the vegetation. The main slope of the ground is to the N.E., a dip of about 2°; there is also a scarcely perceptible one to the N. Owing to this slope, drainage leads away from the area.

A small amount of shelter from certain winds is afforded by rising ground and the National Library.

Grazing by horses or cows is fairly regular during Autumn, Winter and early Spring; hens, crows, pigeons and smaller birds are frequent visitors. Thus, the field may be said to be under conditions of natural manuring. For the remaining months the grass is allowed to grow for hay.

The vegetation is essentially mesophytic and is that of an ordinary pasture field in which the species are not numerous. The dominant plants are *Cynosurus* and *Poa*, which form a compact turf layer with here and there small patches of the coarser grasses, notably *Dactylis glomerata*; a small, low-growing moss, *Eurhynchium* sp., is very common everywhere, and is chiefly abundant on the clover patches. Weeds are scattered unevenly over the field.

The following list gives a relative scale of frequency for the main plants belonging to this area; the number 10 denoting the dominant

forms. *Cynosurus cristatus* 10; *Poa annua* 10; *P. pratensis* 10; *Eurhynchium* sp. 10; *Ranunculus bulbosus* 8; *Cynosurus echinatus* 7; *Poa trivialis* 7; *Dactylis glomerata* 7; *Trifolium repens* 7; *Ranunculus repens* 5; *Cerastium vulgatum* 5; *Urtica dioica* 5; *Rumex crispus* 4; *Carduus pratensis* 4; *Veronica Chamaedrys* 4; *Bellis perennis* 4; *Trifolium pratense* 3; *Chrysanthemum leucanthemum* 3; *Euphrasia officinalis* 3; *Trifolium incarnatum* 2; *Rumex acetosa* 2; *R. acetosella* 2; *Ranunculus acris* 2; *Taraxacum-dens-leonis* 2; *Plantago lanceolata* 1.

High S.W. winds are very characteristic of the district, not infrequently in the form of gales; these usually result in increased evaporation of moisture from the soil, although being typically "wet" winds, there is less danger from this than might be expected. Probably a certain amount of sea spray is carried but not enough to cause any marked difference in the type of animal or vegetable life.

Precipitation is fairly high, mainly brought by these S.W. winds.

The *water content* of the soil plays a great part both in animal and vegetable life; success and healthy existence depend largely on the condition that water shall be present in an available form(4). It has been found that hygroscopic water is the most essential for plants and capillary water for animals; excess of gravitation water blocks the air spaces and causes the death of both plants and animals. Owing partly to the porous and open nature of the soil in the areas under investigation and the shaley substratum, excess of water drains away to a great extent. On the other hand, drought prolonged for more than a very short time is usually fatal; for example, *Collembola* almost entirely disappear in a long dry period, although they quickly multiply shortly after the return of rain. Animals, however, which are able, usually burrow during a period of drought to a greater depth where moisture lingers for a longer time.

Estimations of the available water content of the soil have been carried out throughout the investigation and the results plotted. This water content will naturally vary in different parts of the field according to the soil components and every slight change in slope.

To obtain an appropriate sample, the soil was taken near the line along which the faunal investigation was carried out, and in order that the samples might be representative, a thin slice of earth of about six inches depth was taken from each of several spots and these were placed together in an air-tight jar, thoroughly mixed and the available water content calculated.

The water content percentages obtained are shown graphically in Fig. 2 together with curves for the soil population. The figure shows that

the percentages recorded vary within considerable limits in a comparatively short space of time. Neither water content nor faunal samples were taken at a particular period during December 1920, when a fairly heavy fall of snow occurred which froze into a hard mass; the top two inches or so of the soil were also frozen, the temperature at one time being 2° C. below zero. The next sample taken after this period showed a marked fall in the totals for the fauna.

The maximum water content also varied with every slight difference in the components of the soil and it was found to increase in direct proportion to the amount of humus present. The same method was adopted as above to obtain a representative sample, though even so, the percentages obtained differed within considerable limits. The average, however, worked out to 81·5 per cent.

The pore space of the soil (average 48·9 per cent.) varied likewise, as it is affected by the presence of organic and colloidal matter(16) besides being initially dependent on the packing of soil particles of different sizes. On the other hand, the results of estimations for hygroscopic moisture showed no marked variability, giving an average percentage of 3·45.

The humus content of the soil varied with depth:

1–3 inches depth	14·2	per cent.
3–6 ,,	14·5	,,
6–9 ,,	10·0	,,

Throughout the greater part of the investigation, weekly temperature readings were taken in the atmosphere (in the shade) and at depths of $4\frac{1}{2}$ and 9 inches. The data recorded show the following points:

i. That there is a decided difference between temperatures at different depths.

ii. That the temperature of the soil varies in accordance with that of the atmosphere. The two periods at which the atmosphere and soil curves separate notably from one another are marked by very sunny days.

iii. That the soil temperature varies less than that of the atmosphere, the latter being higher than that of the soil on warm days and lower on very cold days.

iv. That, generally speaking, the soil at 9 inches depth is warmer than that at $4\frac{1}{2}$ inches. Consequently the fauna would find a certain advantage in burrowing deeper into the soil during excessively cold periods.

A mechanical analysis of representative samples of the soil gave the following table of results:

	Maximum and minimum size of particles mm.	Percentage
Fine gravel	3·1	22·36
Coarse sand	1–0·2	15·96
Fine sand	0·2 –0·4	13·34
Silt	0·04 –0·01	12·80
Fine silt	0·01 –0·002	18·60
"Klay"	0·001–below	11·88
Loss on treatment with HCl		3·25.
Hygroscopic moisture		3·45.

Full chemical analyses were not made, but it was apparent that the amount of carbonates in the soil was negligible; that iron was present to a marked degree, and that lead though present in the district was not detected in this soil by the ordinary tests.

(ii) The soil fauna. The term Soil Fauna is used to include all members of the animal kingdom, other than microscopic forms, occurring actually within the soil. Any forms found on the surface are included providing they may also occur beneath the soil, e.g. *Collembola*, *Coleoptera*, and so on.

Representatives of the following divisions were present: *Nematoda*, *Oligochaeta*, *Crustacea*, *Arachnida*, *Myriapoda*, *Insecta*, *Mollusca*.

Of the above, the sub-groups *Collembola* and *Acarina* were the best represented in point of numbers of individuals, and the *Coleoptera* in variety of species.

When following the quantitative changes in the fauna of an area under varying conditions throughout the year, it is important to attempt some estimate of any statistical error that may be involved by the method of collecting the data. One requires some knowledge as to the horizontal distribution of the fauna, and some indication as to whether the standard sample taken is of sufficient size. An effort has been made to test the value for quantitative purposes, of the data presented, in the following way.

i. On each of several occasions 20 small samples of soil, each a cube of two inches, were taken at random from all over the field, the only condition being an apparently similar covering of vegetation in each case. The number of *Achorutidae* present were counted for each sample, as an index of the total census.

The results showed a great range in numbers within each series of 20 countings, being for example in one series from 5 to 22, and one may conclude that a cube of two inches is an unsatisfactorily small sample. However, the totals of the several series (192, 193, 194, 196, 199, 199, 201, 204, 204) were comparable, suggesting that a cube of 9 inches is a sufficiently large sample.

ii. Similar two-inch cubes of soil were taken according to a methodical plan, namely along parallel lines, running N.E. to S.W., at a distance of 20 feet from each other, the samples being spread along the lines 12 feet apart.

The totals for these series agreed very well with the totals obtained above (194, 194, 194, 195, 195, 197, 198, 200, 200), the individual countings for each small sample as before showing a wide range.

A series of similarly placed samples from lines running N.W. to S.E. gave totals 179, 190, 190, 190, 194, 196, 196, 199, 199. Only in the first total above, of all the series taken, was there an absence of high counts.

iii. Lastly, 20 two-inch cubes were removed from the surface of three normal nine-inch samples. Once more the high variability of the individual counts was noted, and totals comparable with those obtained previously (201, 201, 204).

The totals of all these series of samples range as follows: 179, 190, 190, 190, 192, 193, 194, 194, 194, 194, 194, 195, 195, 196, 196, 196, 197, 198, 199, 199, 199, 199, 200, 200, 201, 201, 201, 204, 204, 204, and if one omits the first of these the similarity of the others is evident.

It was concluded that the examination of a nine-inch cube of soil might be expected to give a reasonable picture of the Achorutid population of the soil as a whole and that such a sample was a reasonable one on which to work in attempting to trace quantitatively the seasonal changes of the fauna. Samples of this volume were examined from June 1920 to October 1921, inclusive, but for the remaining months it was thought to be sufficiently instructive to deal with the surface three inches of soil only.

The census of the fauna obtained throughout the investigation is set out in the following tables and some of the data from these tables are summarised and represented graphically by the curves in Figs. 3 and 4. The columns in Fig. 4 show the relative frequency of occurrence of the individual groups.

Specific distribution of the soil fauna.

The Seasonal Census.

The formula used in the following list is based on that used by Morris (20). For example:

June 1920–1.

Lumbricus terrestris $\frac{\text{A } 72 : 10\ (8)}{6, \text{ etc. to } 5} : \frac{\text{B } 14}{7, 8, 10, 11, 1, 5}$.

A = surface three inches of soil: 72 = total number of individuals found in the samples examined between June 1920 and June 1921 (average—two samples per month): 10 = the maximum number of individuals which occurred in any one sample: (8) = the month in which this maximum occurred, that is, August. Below the line "6, etc. to 5" indicates the months in which *L. terrestris* was found. That is, in every month in which samples were examined. Similarly, *B* represents the remaining layers of soil in the samples, that is, from three to nine inches depth: 14 = the total number of individuals found therein: "7, 8, 10..." the months in which they occurred, that is July, August, October.... As the total numbers are so much smaller in B, the maximum total in any one sample has not been included.

The letter (or letters) in brackets placed after the name of a species, e.g. *Dilophus Febrilis* L. (L) indicates the stage of development, *i.e.* (L) = Larva or (LPA) = a species found in larval, pupal, and adult stages, or (C) = cocoon.

Distribution of the soil fauna of pasture land.

[Average—2 samples per month]

	June 1920–1 (Sept. and Dec. not included)		June 1921–2 Surface 3 ins. only*
Nematoda			
Nematoda spp.	A 321 : 139 (10) / 6, 7, 8, 10, 11, 4, 5	: B 32 / 6, 7, 10, 11	346 : 56 (12) / 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
Mermithidae including Mermis brevis Hagmeier	A 15 : 4 (11) / 7, 10, 11, 1, 3, 5	: B 46 / 6, 7, 8, 10, 11, 1, 4	6 : 3 (4) / 3, 4, 5
Oligochaeta			
Small white forms including Fredericia unibaelsenia, F. diabeta, F. bisetosa Ler., F. perrieri	A 593 : 86 (10) / 6, etc. to 5	: B 51 / 6, 7, 8, 10, 11, 1, 2, 4, 5	43 : 8 (1) / 6, 9, 10, 11, 12, 1, 2, 3, 4
Enchytraeids including E. albidus, etc.			
Lumbricus terrestris	A 72 : 10 (8) / 6, etc. to 5	: B 14 / 7, 8, 10, 11, 1, 5	80 : 14 (9) / 6, 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
L. costaneus	A 3 : 2 (2) / 1, 2	: —	1 / 1
Helodrilus oculatus	A 12 : 4 (8) / 8, 10, 11, 1	: B 1 / 6	2 / 2, 5
Allolobophora chlorotica	A 8 : 2 (10) / 6, 7, 8, 10, 11, 1	: —	15 : 4 (1) / 8, 9, 1, 3, 4

* For five months of this year samples were examined to a depth of 9 inches (see Fig. 2), but for convenience the population of the first 3 inches only is given for June 1921–2. So that in the text the total numbers refer to the figure rather than the tables.

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)		June 1921–2 Surface 3 ins. only
ISOPODA			
Porcellio scaber	A 1 3	: —	1 7
ARACHNIDA			
(*a*) *Acarina*			
(1) Gamasidae			
Pergamasus crassipes Linn.	A 127 : 22 (11) 6, etc. to 5	B 15 : 7, 10	156 : 40 (12) 6, 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
var. longicornis	A 3 8	: —	—
P. septentrionalis Oud.	A 29 : 7 (11) 11, 1, 3, 4, 5	: —	11 : 2 (11, 4, 5) 8, 11, 1, 2, 4, 5
P. rumiger Berl.	A 1 11	: —	—
P. meridionalis Berl.	A 1 11	: —	—
P. runcatellus Berl.	A 1 2	: —	—
Oligogamasus pollicipatus	A 21 : 7 (1) 11, 1, 2, 3, 5	B 1 : 5	10 : 3 (10) 7, 10, 11, 1, 2, 3, 5
Gamasus fimetorium Berl.	A 7 : 2 (8, 10) 6, 7, 8, 10, 1	B 1 : 10	20 : 10 (10) 6, 10, 11, 1
G. magnus Kr.	A 13 : 4 (11) 10, 11, 4, 5	: —	10 : 3 (3, 5) 3, 5
Pachylaelops sp.	A 34 : 10 (11) 10, 11, 1, 2, 3, 4, 5	B 4 : 10, 1, 5	52 : 9 (1) 7, 8, 9, 11, 12, 1, 2, 3, 4, 5
Zercon perforatulus Berl.	—	: —	3 : 2 (1) 1, 3
Dinychus tetraphyllus Kr.	A 1 4	: —	4 : 3 (2) 1, 2
Spp. Nymphs	A 61 : 10 (10) 7, 8, 11, 1, 4, 5	B 5 : 7, 10	298 : 34 (1) 6, 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
(2) Tyroglyphidae			
Rhizopus echinopus Rob.	A 12 : 4 (8) 8, 10, 11, 4, 5	B 1 : 10	11 : 3 (1) 11, 1, 3, 5
(3) Thrombidiidae			
Bdella lignicola Can.	A 3 : 2 (11) 11, 1	: —	—
B. lacustris Hull.	A 3 4, 5	: —	6 : 3 (6) 6, 9, 5
Enemothrombium subrasum Berl.	A 5 : 2 (11) 7, 10, 11, 1	: —	1 11
(4) Oribatidae			
Archipteria redux Hull.	A 1067 : 150 (6) 6, etc. to 5	B 51 : 7, 8, 10, 4	1893 : 184 (5) 6, etc. to 5
Notaspis lacustris Michael	A 4 6	: —	5 : 3 (9) 7, 9, 1

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)		June 1921–2 Surface 3 ins. only
ARACHNIDA (*contd.*)			
(*b*) *Araneae*			
(1) Ciniflonidae			
Ciniflo atrox	A 4 : 2 (5) / 1, 5	: —	5 : 3 (5) / 5
(2) Linyphidae			
Walckenaera punctata	A 5 : 3 (1) / 10, 1, 5	: —	3 : 2 (6) / 6
W. pratensis	A 1 / 10	: —	3 : 2 (6) / 6
(3) Epëiridae			
Epëira diadema	—	: —	1 / 7
MYRIAPODA			
(*a*) *Symphyla*			
Including spp.: Scolopendrellopsis subnuda Hans. / Symphylella delicatula Bagn. / S. vulgaris Hans.	A 33 : 12 (5) / 6, 8, 10, 11, 1, 2, 3, 4, 5	: B 106 / 6, etc. to 5	55 : 9 (5) / 6, 7, 8, 11, 2, 4, 5
(*b*) *Diplopoda*			
Julus varius	A 1 / 1	: —	—
J. sp.	A 2 / 5	: —	—
Sp.	—	: —	1 / 11
(*c*) *Chilopoda*			
Geophilus longicornis Leach	A 8 : 2 (2) / 7, 11, 1, 2, 5	: B 8 / 7, 8, 10, 11, 1	2 / 1, 5
Lithobius erythrocephalus	—	: B 1 / 6	—
L. forficatus	—	: B 1 / 2	—
Brachygeophilus truncorum	—	: B 2 / 1	—
INSECTA			
(*a*) *Collembola*			
(1) Achorutidae			
Onychiurus ambulans Linn.	A 212 : 45 (2) / 6, etc. to 5	: B 148 / 6, etc. to 5	1288 : 155 (4) / 6, etc. to 5
O. armatus (Tulb.)	A 343 : 123 (11) / 6, etc. to 5	: B 209 / 6, etc. to 5	318 : 49 (11) / 6, etc. to 5
O. fimetarius Linn.	A 131 : 58 (11) / 7, 10, 11, 1, 2, 3, 4, 5	: B 62 / 6, 8, 10, 11, 1, 3	5 : 4 (1) / 6, 1
O. Burmeisteri (Lubb.)	A 11 : 7 (2) / 2, 4	: B 5 / 2	1 / 7
Achorutes armatus (Nic.)	A 10 : 3 (2, 5) / 8, 10, 2, 5	: B 1 / 2	905 : 488 (3) / 6, 7, 9, 10, 11, 1, 2, 3, 4, 5

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)		June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)			
A. purpurescens Lubb.	—	—	1 11
Tullbergia quadrispina (Börn.)	—	—	47 : 23 (11) 11, 1, 2, 3, 4, 5
(2) Entomobryidae			
Entomobrya muscorum (Nic.)	A 5 : 2 (11, 4) 11, 4	—	23 : 12 (8) 8, 1, 4
E. multifasciata (Tulb.)	A 6 : 4 (4) 11, 4	B 1 2	1 3
E. Nicoleti (Lubb.)	A 33 : 10 (11) 11, 1, 3, 4, 5	B 2 1	20 : 8 (5) 8, 9, 11, 5
Isotoma viridis Bourl.	A 260 : 125 (2) 6, 7, 10, 11, 1, 2, 3, 4, 5	B 9 11, 1, 2, 3, 4, 5	854 : 139 (1) 6, etc. to 5
I. plumbea Lubb.	A 2 1	—	1 5
I. anglicana Lubb.	A 10 : 9 (2) 1, 2	—	2 2, 3
I. grisescens Schäff.	A 4 7	B 1 7	3 11
I. viatica Lubb.	A 1 1	—	1 6
Isotomurus palustris (Müll.)	A 80 : 18 (11, 1) 7, 8, 10, 11, 1, 2, 3	—	35 : 12 (5) 1, 2, 3, 4, 5
var. prasina Reut.	A 16 2	—	—
Beckia argentea Lubb.	A 13 : 5 (10) 8, 10, 11, 1, 4	B 2 1, 2	—
B. albinos Nic.	A 2 10, 1	—	5 8
Folsomia fimetaria Tulb.	A 51 : 32 (2) 6, 7, 8, 1, 2, 4, 5	B 14 1, 2, 3	716 : 145 (5) 6, etc. to 5
(3) Sminthuridae			
Sminthurus viridis Lubb.	A 10 : 6 (5) 2, 5	B 5 2, 5	8 : 3 (7) 6, 7, 9, 1, 5
S. aureus var. ochropus Reut.	A 2 11, 5	—	—
S. luteus Lubb.	A 3 : 2 (11) 11, 1	B 1 5	20 : 12 (6) 6, 3, 4, 5
S. niger (Lubb.)	A 9 : 2 (4, 5) 11, 4, 5	—	4 : 2 (11) 11, 4
Bourletiella hortensis (Fitch.)	A 1 6	B 1 8	2 6, 7
*"Larvae"	A 160 : 29 (10) 8, 10, 11, 1, 3, 4, 5	B 77 6, 7, 10, 1, 2, 3, 4, 5	1725 : 181 (5) 6, 9, 10, 11, 12, 1, 2, 3, 4, 5
(*b*) *Rhynchota*			
(1) Heteroptera			
Calcoris sp. (N)	A 1 6	—	—

* The arbitrary term ' Collembolan larvae" is used to include such forms of the group as are too immature for specific identification.

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)				
(2) Homoptera				
Myzus sp. (AN)		—	: —	14 : 10 (10) / 7, 9, 10, 2, 5
Sp. (N)	A 2 / 2		: —	6 : 2 (7, 8) / 7 8, 11
(3) Psyllidae				
Psylla sp. (A)	A 1 / 2		: —	—
(*c*) *Thysanoptera*				
Physothrips consociata (A)	A 2 / 6		: —	1 / 5
(*d*) *Lepidoptera*				
Spp. (3) (L)		—	: —	3 : 2 (11) / 11, 5
(*e*) *Coleoptera*.				
(1) Carabidae				
Amara communis Panz. (A)	A 4 / 7, 11, 1, 2		: —	1 / 9
Bradycellus verbasci Duft. (A)	A 1 / 11		: —	—
Nebria brevicollis F. (L)	A 7 : 2 (7, 10) / 7, 10, 11, 1		: B 1 / 1	2 / 8
Spp. (3) (L)	A 1 / 2		: —	7 : 6 (6) / 6, 11
(2) Hydrophilidae				
Megasternum boletophagum Marsh (A)	A 1 / 5		: B 1 / 4	3 : 2 (9) / 6, 9
(3) Staphylinidae				
Xantholinus linearis (A)	A 2 / 10, 5		: B 1 / 10	19 : 5 (10, 5) / 7, 9, 10, 11, 1, 3, 4, 5
X. sp. (L)	A 2 / 10		: —	4 : 2 (6) / 6, 5
Ocypus olens Müll. (A)		—	: —	1 / 11
O. cupreus Rossi. (A)	A 3 : 2 (6) / 6, 7		: —	2 / 5
Philonthus or Quedius sp. (L)	A 2 / 6, 1		—	17 : 6 (5) / 6, 7, 11, 1, 3, 4, 5
Homalota fungi Gr. (A)		—	: —	1 / 9
Spp. (2) (L)	A 1 / 5		: —	5 : 3 (11) / 11, 2
(4) Pselaphidae				
Euplectus kunzei Aubé (A)		—	: —	1 / 7
(5) Trichoptergidae				
Trichopteryx sp. (A)	A 3 / 6, 1, 3		: B 1 / 8	1 / 8
Ptenidium evanescens Marsh (A)	A 1 / 6		: —	—

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)				
(6) Byrrhidae				
Byrrhus pilula L. (L)	A 8 : 3 (11, 5) / 11, 2, 5	:	—	1 / 4
(7) Lathridiidae				
Corticaria fulva Com. (A)	A 1 / 5	:	—	—
(8) Lucanidae				
Sp. (L)	A 1 / 2	:	—	—
(9) Scarabaeidae				
Aphodius fossor L. (A)	—	:	—	1 / 1
Aphodius punctatosulcatus Sturm (A)	—	:	—	1 / 11
(10) Elateridae				
Agriotes obscurus L. (L)	A 45 : 6 (4) / 6, 7, 10, 11, 1, 2, 3, 4, 5	: B 1 / 5		26 : 6 (12) / 6, 8, 11, 12, 1, 2, 3, 5
A. lineatus L. (L)	A 6 : 2 (7) / 6, 7, 10, 11	:	—	—
Athous haemorrhoidalis F. (L)	A 3 : 2 (7) / 7, 8	:	—	—
(11) Chrysomelidae				
Longitarsus luridus Scop. (A)	A 1 / 3	:	—	—
L. gracilis Kuts. (A)	A 1 / 5	:	—	—
(12) Crioceridae				
Lema melamopa (A)	A 2 / 6	:	—	—
(13) Curculionidae				
Sitones flavescens Marsh (A)	—	:	—	1 / 6
S. hispidulus F. (P)	A 1 / 2	:	—	1 / 5
Hypera punctata F. (A)	—	:	—	2 / 8, 5
Rhinonchus pericarpius L. (A)	—	: B 1 / 5		1 / 11
Lissoma ovatulum Clairv. (A)	—	:	—	1 / 11
Sp. (L)	A 13 : 3 (4, 5) / 11, 1, 2, 3, 4, 5	: B 1 / 5		57 : 7 (11) / 6, 7, 8, 9, 10, 11, 1, 3, 4, 5
(14) Helopidae				
Helops strictus (L)	A 1 / 2	:	—	—
(*f*) *Diptera*				
(1) Cecidomyiidae				
Cecidomyia buxi (P)	A 1 / 6	:	—	—
C. destructor Say. (P)	A 2 / 5	:	—	—

[Average—2 samples per month]

	June 1920–1 (Sept. and Dec. not included)		June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)			
C. sp. (L)	A 4 : 2 (1) / 1, 5	: —	2 / 6
(2) Mycetophilidae			
Sciara sp. (L)	A 17 : 6 (2) / 11, 1, 2, 4	: —	—
(3) Bibionidae			
Dilophus febrilis L. (L)	A 132 : 96 (1) / 11, 1, 3	: —	71 / 1
Bibio Johannis L. (L)	A 1 / 2	: —	—
(4) Tipulidae			
Tipula paludosa Mg. (L)	A 10 : 2 (1, 3) / 10, 1, 2, 3, 5	: —	15 : 5 (10) / 8, 9, 10, 11, 2, 5
T. sp. (L)	—	: —	15 : 5 (4) / 1, 2, 3, 4, 5
(5) Leptidae			
Leptis scolopacea L. (L)	A 39 : 9 (11) / 6, 7, 8, 10, 11, 1, 5	: B 6 / 6, 7, 10, 11	6 : 2 (9) / 9, 10, 1, 2, 4
L. tringaria L. (L)	A 4 / 11, 1, 2, 5	: B 2 / 11, 1	1 / 6
(6) Empidae			
Rhamhomyia sulcata Fln. (P)	A 2 / 4, 5	: B 2 / 4	—
(7) Cordyluridae			
Scatophaga stercomaria L. (P)	—	: —	1 / 1
(8) Anthomyiidae			
Hyetodesia incana W. (L)	A 20 : 5 (1) / 8, 10, 11, 1, 5	: B 1 / 4	2 / 3, 5
Coenosia sp. (P)	A 1 / 5	: —	—
Hydrotoea irritans Fln. (P)	A 6 : 5 (5) / 4, 5	: —	17 : 3 (5) / 6, 11, 1, 2, 4, 5
(9) Chironomidae			
Tanypus maculatus (L)	A 3 : 2 (1) / 11, 1	: —	—
(10) Phoridae			
Phora sp. (P)	A 4 : 2 (2) / 6, 2, 5	: —	15 : 4 (4) / 7, 8, 11, 12, 1, 3, 4
(11) Spp. (3) (LP)	A 6 : 3 (2) / 11, 2, 3, 5	: B 1 / 6	6 : 2 (9, 5) / 7, 9, 1, 5
(*g*) *Hymenoptera*			
Aphidius danci (C)	A 3 : 2 (6) / 6, 4	: —	—
Myrmica scabrinodis (APL)	—	: —	273 / 7
Pteromalinae-Chalcidae			
Sp. (A)	—	: —	2 / 6, 7

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only	
INSECTA (*contd.*)					
(*h*) *Mollusca*					
Limax agrestis	A 7 / 6, 10, 11, 1, 2, 5	:	—		—
L. sp.	A 3 / 8, 11, 5	:	—	2 / 1, 2	
Arion ater	A 1 / 10	:	—		—
Planorbis sp.	A 1 / 10	:	—	3 / 8	

Fig. 2 shows the quantitative seasonal changes in the total fauna for each of the three specified depths of soil. It is evident that the population of the first three inches is, with rare exception, markedly greater than that of lower depths, and that for the most part, the general rise and fall of the curves is fixed by this "upper" population. The upper curve shows throughout an irregular rise and fall, but in addition it has a well-marked trend, being high in winter and low in summer.

The difference in the levels of the curve during summers 1920 and 1921 is very marked and has every appearance of being related to the unusual precipitations and unusual dryness, respectively, of these two years. The dry conditions of 1921 certainly caused many organisms to die off, their shrivelled bodies being found in considerable quantities.

Points on the curves marked *, †, ⊙, ⁂, × indicate the occurrence of large colonies of organisms which are not included in the totals from which the curve was drawn, as they were not typical of a sample, but only occurred scattered here and there over the field. These colonies did not appear to have any influence upon the rest of the fauna or flora of their immediate neighbourhood.

The census curves for the depths three to six inches and six to nine inches follow each other very closely; their respective populations do not materially differ and the numbers are small. In no case does the population of either of these lower depths exceed that of the surface three inches. Just as the curve for the total fauna is determined by the fauna of the upper three inches, so that for the upper three inches is itself determined by the prevalence of *Collembola* and *Acarina* (Fig. 3).

The soil was examined, occasionally, below the specified depth of the routine samples. Under normal or very moist conditions organisms were of very rare occurrence below nine inches; an occasional *Achorutid*

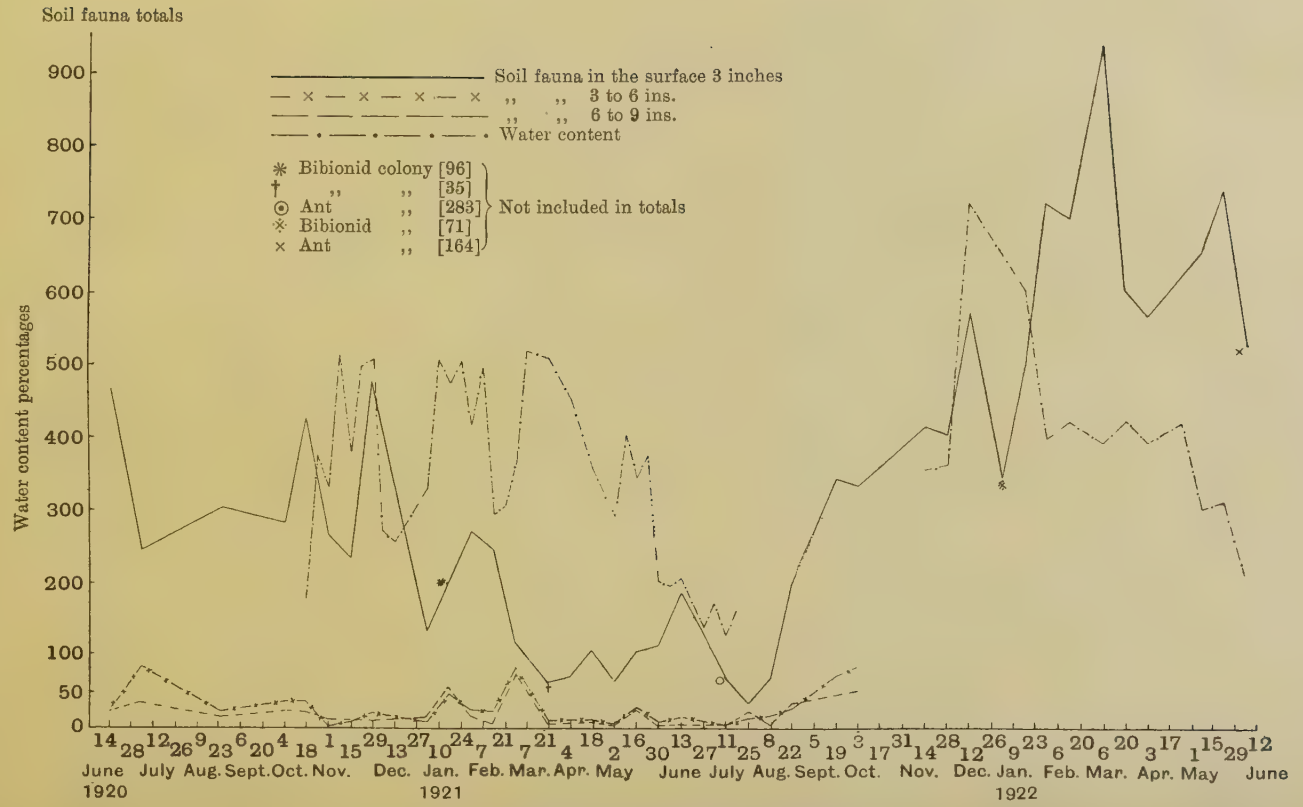


Fig. 2. Distribution of the soil fauna—Pasture land.

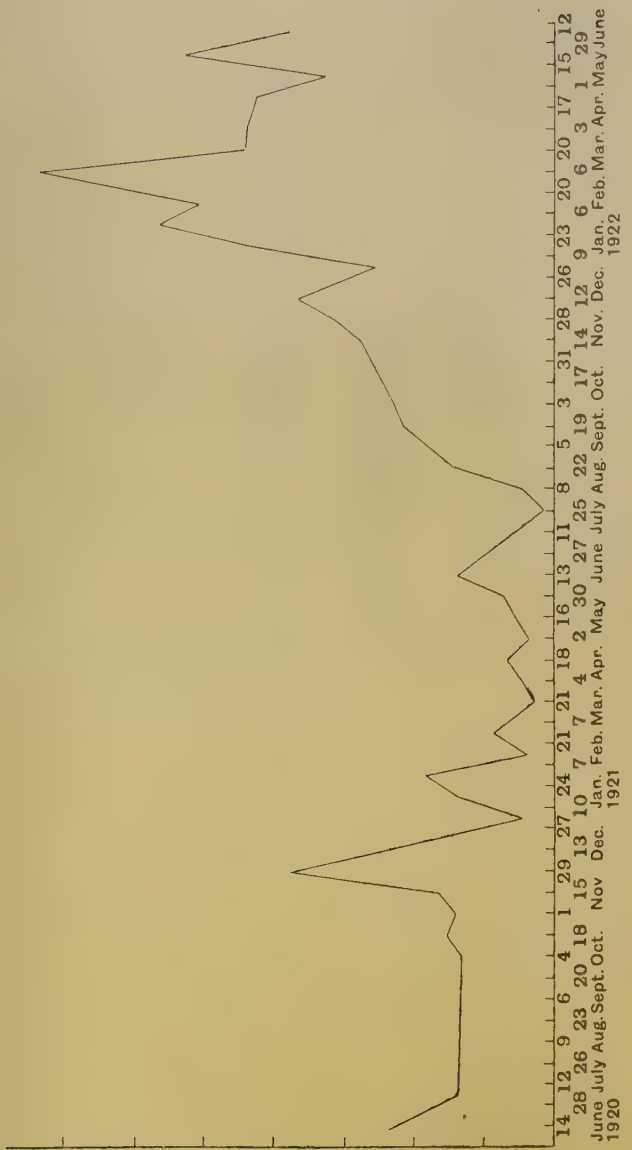


Fig. 3. Curve of the totals of Achorutidae, Collembolan larvae and Acarina [in the surface three inches of soil].

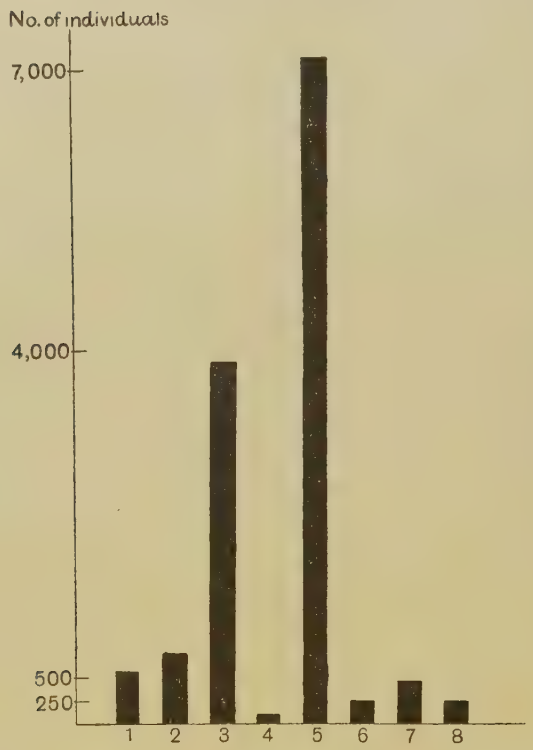


Fig. 4. *Pasture.* Relative frequency of occurrence of individual groups in the surface three inches of soil.

Scale—1 cm. =500 individuals.

1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera; 7, Diptera; 8, Hymenoptera.

Totals of other groups are too small to be included in the diagram.

was found. As the soil became dry, *Oligochaetes* began to work their way downwards, and when drought conditions held, a few *Symphyla* and *Achorutidae* and an occasional insect larva occurred at a depth of from nine to twelve inches, but not lower.

The curve for the water content of the soil has been included in Fig. 2; it is based on the percentages occurring either weekly or on the days on which the samples were taken. There is some suggestion that the water content of the soil may offer an explanation of the variation of the faunal census, though this is by no means proved. Certainly the effect of moisture on *Collembola* and small white *Oligochaetes*, at least, is very marked.

Of the *Insecta* the group *Collembola* (17) was most abundantly represented in the soil fauna as regards the number of individuals and showed a fairly wide variety in species. They were ubiquitous, occurring vertically and horizontally throughout the samples. Whilst far more numerous in the upper three inches, it is rather surprising that a considerable number of these delicate organisms should occur at comparatively low depths. In these lower parts of the soil the majority of the forms found were representatives of the *Achorutidae*, in which family the saltatory apparatus is poorly developed or missing. Less frequently "springing" forms were also present, though in this position the "spring" could not function in the usual manner.

The number of individuals per sample varied very considerably, for example, from 5 to 543 in the upper three inches. Very low numbers were not common, and when these occurred, they followed a spell of exceedingly cold or very dry weather.

Experiments were carried out with regard to the length of Collembolan life under artificial drought conditions. A number of healthy adult *Achorutidae* were placed in a shallow dish, the water content of the soil being approximately 36 per cent. The dish was left exposed to the air of the laboratory, the temperature of which was 40° F. The soil dried in the night, leaving only a certain amount of hygroscopic moisture. On examination of the organisms on the following day less than 50 per cent. were found alive and all had succumbed by the third day.

The extremely small number of individuals, five only, found in the sample January 3rd, 1921, might be explained as being related to the preceding severe cold and low water content during December 1920, for at the end of November, the numbers reached 252. There was again an increase to 149 on January 31st, 1921, consisting of larvae and adults. This seems to imply that a new generation had appeared, hatched from eggs which had resisted the inclement conditions. According to Lubbock (17) "the eggs are laid and the young hatched apparently within a period of from six to ten days."

The food of these Collembolans seemed to be humus, though a few specimens were noted which appeared to be nibbling the turf roots. As stated by Theobald (27), they can do sufficient damage to open up plant tissues to fungal or bacterial attack; those species which feed on the attacking fungi or bacteria are probably carriers of the disease. Species known to be injurious and which occurred in this area are as follows: *Onychiurus ambulans*, *O. armatus*, *O. fimetarius*, *O. Burmeisteri*, *Achorutes*

armatus, *A. purpurescens*, *Isotomurus palustris*, *Entomobrya multifasciata*, *Sminthurus luteus*, *Bourletiella hortensis*. No damage to the vegetation, however, has been recorded in the present investigation, and in no case was there evidence of attack by Collembolans on animal forms.

The most common form of parasitic attack upon Collembolans was that by small Nematodes, which pierced the abdomen, usually between the second and third segments. In some cases the Nematodes were wholly internal, coiled round the organs, but when large they appeared to penetrate the organs, leaving half the body projecting freely from the host. Throughout the time they were under observation the position of the parasite did not alter and the hosts were capable of slow dragging movements. In the cases noted the attack was confined to members of the *Achorutidae*.

Bacteria were present in the majority of specimens examined, apparently not harmful types.

Ecdyses of the *Collembola* were evidenced by the presence of perfect specimens of whitish cast skins in the soil at varying depths. With two exceptions, they occurred in each month during the course of the investigation, being found mainly in the upper three inches of soil. The maximum numbers of cast skins occurred in June 1920, September 1921, January, April and May 1922.

The three families *Achorutidae*, *Entomobryidae* and *Sminthuridae* were represented by 7, 12 and 5 species respectively. Four of the 24 species, however (*Achorutes armatus*, *Onychiurus ambulans*, *O. armatus* and *Isotoma viridis*) contributed approximately 58·5 per cent. of the total number of adults (7176) found during the investigation in the first three inches of soil. In the lower layer of soil, the fall in numbers was very marked; this is probably due to the concentration of the food supply in the first two or three inches of soil (humus and roots) and possibly the better aeration of the soil near the surface. The species in the lower layer were similar to those in the upper though with less variety in the *Entomobryidae*.

Owing to the relative inactivity of the *Achorutidae* and the considerable numbers in which they occur, these organisms form excellent material for quantitative study. The family contributed the greater part of the Collembolan population at all depths in the soil. In no case did they appear to form definite colonies, but were fairly evenly distributed.

Entomobryids contributed but little to the composition of the fauna at depths below three inches; of the 12 species recorded, *Isotoma viridis*

was by far the most abundant, varying in size and colour to a great extent. Since the springing organ in this family is strongly developed, some individuals may have escaped during the collection of the samples, but it is thought that on the whole, the figures convey a fair idea of quantitative occurrence.

Throughout the investigation it was noted that *Isotoma viridis* and *Isotomurus palustris*, or *Isotoma viridis* and *Folsomia fimetaria*, were usually found together, and *Onychiurus ambulans* and *O. armatus* almost invariably so.

The *Sminthuridae* were at all times of rare occurrence and were found typically among the bases of grass tufts. They appeared to feed on living vegetation though were not present in sufficiently large numbers to cause any perceptible damage. The largest form, *Sminthurus viridis*, was the most common species.

Larvae contributed approximately 23 per cent. of the total Collembolan individuals.

The group *Rhynchota* was poorly represented and the individuals which occurred belonged mainly to the *Homoptera*. *Aphid* nymphs were the most frequent, a few individuals being sufficiently advanced to be referred to the genus *Myzus*. One individual appeared to represent a new species of this genus. The only *Heteropteran* present was a nymph in a very early stage of development; probably *Calcoris* sp. With one exception, the above individuals all occurred immediately beneath the surface.

Representatives of the *Thysanoptera* and *Lepidoptera* occurred very rarely, seven specimens only being found in the total number of samples.

Coleopterans (11, 22) were fairly well represented in point of species (35) though the total number of individuals recorded (280) was not great. The only species found with any frequency were *Agriotes obscurus* and *Weevil* larvae. Together these contributed a very considerable proportion of the total *Coleopteran* population. Recognised pests were present, notably *A. obscurus* and *Weevil* larvae, but no particular damage was noted for this area.

Representatives of 14 families occurred, adults and larvae, but the latter were always more frequent. Owing to the difficulty of rearing *Coleopterous* larvae to the adult stage for identification, many cannot be referred to more than their genus or even family. The larvae occurred almost exclusively within the upper three inches of soil; this is probably explained by the fact that the majority are root feeders and the level of the root area ends, for the most part, $2\frac{1}{2}$ to 3 inches down. Carnivorous forms would also tend to concentrate here.

The larva of *Nebria brevicollis* was the most commonly occurring form, usually found in connection with the Elaterid *Agriotes obscurus*. One individual was captured holding two small specimens of *Onychiurus ambulans* between its mandibles, tenaciously clinging to them even when disturbed; these may or may not form part of their regular diet.

Amara communis may be merely a temporary invader of the area, as the adults show a marked habit of flying from place to place and only penetrate the soil very superficially; also, no larvae were found.

A rare form recorded was *Euplectus kunzei*.

More species were recorded for the *Staphylinidae* than for any other family (8), *Xantholinus linearis* adults and *Quedius* (or *Philonthus*) sp. larvae being the most common, both of which appeared in the lower layers of soil as well as in the upper.

The Criocerid *Lema melamopa* is interesting in that while it often occurs on the leaves of rye grasses and other Gramineae, in this investigation specimens were found free in the soil; possibly the eggs may be laid underground.

Larval forms of *Curculionidae* were fairly abundant and usually found round the roots of *Dactylis glomeratas*.

Diptera (28, 29) were well represented (20 species) though much less so than the previous group. The total number of individuals was, however, increased by the occasional occurrence in the samples of large colonies of larvae of the Bibionid *Dilophus febrilis*; these occurred in three samples and gave totals of 96, 35 and 71 individuals respectively. When reared the adults usually emerged two months earlier in the laboratory than they would have done under normal conditions.

Tipulid larvae appeared to feed on all roots irrespective of species and larvae of *Leptis scolopacea* exclusively on humus or the rotting bases of grass sheaths or leaves, though its family the *Leptidae* is notoriously predaceous.

Tanypus maculatus was remarkable in its occurrence in normal pasture land, it being typically found in water; here it occurred over 200 feet from the stream traversing the neighbouring pasture field. All the characteristic features were fully developed. During summer months, the adults of this family were often found entangled among the grasses of the samples as were also those of *Phoridae* and *Cecidomyiidae*; only the immature forms of the families are, however, included in the census.

The group *Hymenoptera* was not well represented in the samples, the most notable instances being the occurrence of large colonies of *Myrmica scabrinodis* in adult, pupal and larval stages in from one to six inches

depth of soil. The colonies did not appear to have any disturbing effect on the rest of the population of the sample.

Two small adult individuals of the sub-family *Pteromalinae* (Chalcidae) were found beneath the surface; it has not been possible to identify them.

Specimens of *Aphidius danci* were taken from the soil as tiny cocoon-like structures; the species is a typical parasitic form which undergoes metamorphosis within the dead body of the host—a small Aphid—under which conditions it does not form a cocoon-like pupal covering. Here, however, the specimens were free in the soil and not in any intimate relation with a host. Other species of this genus are known to feed on the roots of plants.

With the exception of the *Collembola*, the *Acarina* (14) contributed the greatest number of individuals towards the soil fauna. These were distributed among four families and, excluding the nymphs, among 17 species.

The organisms occurred at all depths in the soil, but were most common in the surface three inches. They were ubiquitous, being found among the roots of the vegetation, free in the soil and on other soil animals. They appeared to respond to moisture conditions in much the same way as did the *Collembola*. In similar laboratory experiments, it was found that the Oribatid *Archipteria redux* offered the longest resistance to drought conditions, though this did not amount to more than four days in soil with initial water content 36 per cent. and which was allowed to dry at ordinary room temperature.

A. redux appeared to be a typical member of the permanent soil fauna of the field, and itself contributed approximately 77 per cent. of the total Acarine population. It was an extremely common species, occurring throughout the investigation although it is usually characteristic of fairly damp places. They were essentially vegetarian, feeding mainly on the moss amongst the roots and shoots of which they were most prevalent.

The most curious form was the vermilion sac-like and sluggish *Enemothrombium subrasum*, a form usually carnivorous with a preference for *Collembola* and *Tyroglyphidae*. The more usual habitats for *Thrombidiidae* are very damp places or even water.

Archipteria fimetorium typically breeds in manure heaps and is often found on members of the *Carabidae*. It was not infrequently met with in the investigation and there may be some connection with the fact that the field is naturally manured.

Four species of *Spiders* were recorded but were not studied in any detail as it is doubtful whether they were true soil inhabitants, although their eggs may be laid underground and the adults themselves were found among the looser upper fragments of soil by the bases of grass tufts.

Members of each of the three divisions of *Myriapoda* were present, but never in large numbers, and the variety in species was not great. Members of the *Symphyla* occurred most frequently and were distributed throughout the nine inches of soil, the numbers increasing with increase in depth. The individuals have not all been separately and specifically identified, but they appeared to be mainly of the same type, and included the species *Scolopendrellopsis subnuda*, *Symphylella delicatula* and *S. vulgaris*. They were relatively inactive and not very responsive to external stimuli and so far as could be ascertained were exclusively humus feeders. They contributed 86 per cent. of the total Myriapod population. Of the remaining divisions the Centipede *Geophilus longicornis* was the most common, although even this was only occasional.

The *Oligochaeta* usually form a very important section of the soil fauna and were of frequent occurrence during the earlier part of the investigation.

After January 1921 the numbers dropped very considerably. It is possible that the water content of the soil may have some bearing on this fact, but it does not afford a complete explanation.

For convenience an arbitrary division of the forms has been adopted: (i) larger Oligochaetes (*e.g.* Terricolae), (ii) small white Oligochaetes (*e.g.* Enchytraeidae); the members belonging to the latter have not been specifically named.

By far the greater number occurred in the first three inches of soil and it is mainly the larger forms which persisted in the lower depths. In a total of 830 approximately 76 per cent. was contributed by the "small white Oligochaetes" which were almost invariably found entwined round the rootlets of any type of plant. These forms varied in size from one-eighth to three-quarters of an inch in length, a variation which held for every sample in which they occurred. A great number were species of the red-blooded *Enchytraeids*, typically vegetable feeders, rather inert unless disturbed and rarely moving from the food supply. An indication of the type of species in the soil is given by the following list: *Fredericia unibaelsenia*, *F. bisetosa*, *F. diabeta*, *F. perrieri* and the Enchytraeid *Enchytraeus albidus*. Under drought conditions the majority appeared to shrivel and die off.

The "larger Oligochaetes" occurred fairly regularly in the form of the common species *Lumbricus terrestris* in all stages of development, and throughout the nine inches of soil, though predominantly in the upper three inches. The disappearance of this species during the dry summer months 1921 was possibly due to withdrawal to a lower depth than that with which the samples dealt. *Helodrilus oculatus* may be mentioned as being the first record for this species in Wales. It is interesting in being the only Lumbricid with eyespots, which are present for a short time during immaturity.

Several specimens were examined for parasites, but except for the common parasites *Monocystis agilis* and Nematodes in the intracellular portion of nephridia, no foreign bodies were evidenced beyond thick walled fungal resting spores and apparently harmless bacteria. Very occasionally, in the spermathecae, structures were present which were probably Nematode eggs.

No parasites were noted in the small white Oligochaetes so far as examined.

As in the Oligochaetes, the *Nematoda* have been for convenience artificially divided into two groups: (i) the large opaque white forms, (ii) the small colourless transparent forms. Again the greater numbers occurred in the upper three inches of soil, mainly due to the Nematodes which were prevalent during the months June to November, inclusive, 1920; after that date with three exceptions (totals 1, 5 and 1 respectively) they did not re-occur until the end of August 1921. This lapse of time seems rather too long to be due entirely to life cycles.

It is doubtful whether the given census figures are truly indicative of the Nematode population, as they are readily overlooked by reason of their smallness and transparency; they are best detected by floating upon water after sieving the soil, where they betray themselves by wriggling violently.

Many of the forms were Tylenchus-like in appearance, and the majority appeared to be free living in the soil, though there were examples of certain forms living in bulbs, on the bases of grass shoots, while others again parasitised certain of the fauna, as in the case previously recorded for the Collembolan.

The members of the improvised division "larger Nematodes" were found free living in the soil and slightly increased in numbers with increase in depth; typically they were beneath the root level and did not appear to be parasitic in any way, mainly feeding on humus. The forms chiefly belonged to the family *Mermithidae* (e.g. *Mermis brevis*).

Mollusca were very poorly represented and seem to have little importance in the general life of the soil fauna.

So far as could be ascertained there was no definite relationship between the fungi and the vegetation, and very little with the fauna of the soil. The only instances noted were attacks on injured organisms in both cases.

In connection with the fauna, fungal growth was found on beetles and earthworms and on pupae should the soil become waterlogged for more than a very short period. With regard to injured earthworms, it is probable that the attack is not wholly external, but rather due to the growth of the spores near the wound, which are inevitably in the alimentary canal by reason of the soil diet. An injured earthworm was found with a tuft of fungal hyphae covering the wound. Inoculations were made from this into beerwort agar and, after incubation, cultures of the following species were obtained: *Mucor racemosus*, *Absidia spinosa*, *Saccharomyces* sp., *Cladosporium epiphyllum*.

One beetle was captured, *Bradycellus verbasci*, with several strands of hyphae projecting from the head, and from between the abdominal segments. The fungus probably belonged to the genus *Monilia*.

4. COMPARISONS WITH OTHER AREAS INVESTIGATED.

(1) Ungrazed grassland. The area now to be discussed has not been studied from the point of view of the seasonal census as such, but rather so as to form a comparison with the pasture land.

	August, 1920				May, 1920
	Pasture field		Ungrazed grassland		
Group	No. of spp.	No. of individuals	No. of spp.	No. of individuals	No. of individuals
Nematoda	1+	69	1+	64	7
Oligochaeta	10+	86	10+	326	563
Acarina	5	107	6	70	288
Araneae	—	—	1	1	—
Myriapoda	5+	4	8+	40	29
Collembola	8	66	6	757	803
Coleoptera	2	2	9	13	13
Diptera	2	7	1	1	8
Lepidoptera	—	—	—	—	1
Mollusca	—	—	1	2	4
Totals	33+	341	43+	1274	1716

The area is a piece of grassland where the vegetation, save for being occasionally scythed, was entirely undisturbed. It is separated from the

pasture field by a hawthorn hedge raised on a bank. The two fields are essentially similar in physical conditions, the nature of the soil, and the vegetation.

A standard sample was taken on August 25th, 1920, both from the pasture field and from the ungrazed area: the first four columns of the preceding list give some idea of the relative frequency of the species and individuals present.

The population of the ungrazed area shows a marked increase over that of the pasture field, both in number of species and in number of individuals. An analysis of the table shows that as regards individuals this increase is in the main due to the *Collembola* and *Oligochaeta* and to the *Myriapoda* to a lesser extent (see Fig. 5). The *Acarina* is the only group where there is any very perceptible decrease in the ungrazed area, but it is doubtful whether any significance should be attached to this, in view of the high numbers recorded for a sample previously taken in May.

The greater numbers were probably due to three main reasons:

(i) Effective shelter and protection for insects in the long grass—from high winds and enemies.

(ii) The soil was kept in a cool, moist condition for a longer time because of the long grass.

(iii) Trampling by the larger grazing animals causes the soil to become compressed into a compact layer, causing diminution of air spaces and rendering efficient respiration more difficult. The soil of the ungrazed area is thus naturally more "open" than the pasture field.

The relative distribution of the fauna in depth did not materially differ from that of the pasture field, being concentrated in the upper three inches.

The dominant species in both areas were markedly the Collembolans *Onychiurus ambulans*, *O. armatus*, and the Acarine, *Archipteria redux*.

The following list gives the species which occurred in the ungrazed area, but not in the pasture field.

MYRIAPODA: *Brachydesmus superus* var. *mosellans*, *Schlendyla memorensis*, *Banjulus* sp., *Polydesmus collaris*.

COLLEMBOLA: *Megalothorax minimus*, *Bourletiella hortensis* var. *juvenilis*.

COLEOPTERA: *Melolonthus vulgaris*, *Syntonium* sp., *Bembidium obtusum*, *Polystethus* sp., *Enicmus minutus*, *E. transversus*, *Tachyporus chrysomelinus*, *Apion virens*.

DIPTERA: *Drymeia hamata*.

A comparison between samples taken from the ungrazed area in May and August 1920 shows that the total soil population was markedly greater in May (see last column in the previous table). Also the total population in either month exceeded any total per sample recorded for any other area which has been examined.

The greater numbers in May were due to contributions from *Acarina* and *Oligochaeta*; on the other hand, *Myriapoda* and *Nematoda* showed higher totals for August.

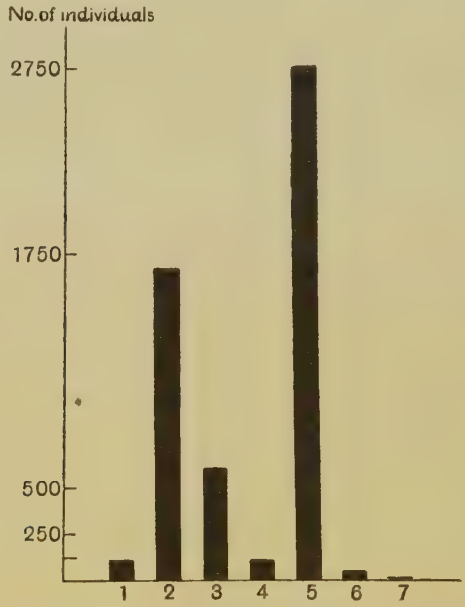


Fig. 5. *Ungrazed Land.* Relative frequency of occurrence of individual groups. Scale—1 cm. = 250 individuals.

1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera; 7, Diptera.

Totals of other groups too small to be included in diagram.

Cast skins of Collembolans were abundant in each month, though varying in distribution, 31 skins being found in the upper three inches and none elsewhere in May, whereas in August one only was found in the upper three inches and 31 in the 6–9 inch layer. These facts are probably in the first place due to changes in the water content of the soil, *i.e.* in August when the soil is normally much drier than in May, the organisms would probably migrate to lower depths for the purpose

of casting their skins, where the water content would be somewhat higher, and there would be less danger of desiccation at a critical period.

One case was noted of parasitism by Nematodes. *Megalothorax minimus* (*Neelidae*) may be mentioned as the only record of this species for the whole investigation; as the form is microscopic it is possibly more prevalent than the numbers appear to indicate.

Species usually associated with dung or refuse were occasional; these may or may not be invaders of the area. They were the Coleopterans *Megasternum boletophagum*, *Enicmus minutus* and *E. transversus*.

(2) LAND RECENTLY PLOUGHED. This adjoined the ungrazed grassland, and was essentially similar in general physical conditions and the nature of the soil.

A standard sample was taken four months after the layer of turf had been removed from the area, and a few days after hoeing. The soil was in a loose open condition and approximated in this respect to the soil of the cultivated land, to be discussed later.

From the treatment of the area four results were inevitable:

(i) That the majority of the organisms should be removed with the turf layer.

(ii) That with the removal of the turf layer the main source of food should also be removed.

(iii) That a certain amount of direct injury would be done to the fauna by ploughing and hoeing.

(iv) That temporary conditions of drought would be set up for a time in the surface layers of soil.

The analysis of the sample is given in the first column of the table on page 380.

Dealing with the May sample, the first noticeable fact was the paucity of the fauna, with the exception of the *Collembola*.

Another marked feature was that the population of the first three inches of soil was *less* than that of the lower levels, the numbers increasing with depth.

In the Collembolan group only *Achorutids* were present; *Coleoptera*, *Diptera*, *Acarina* and *Myriapoda* were very poorly represented and Nematodes were absent. The Chilopod, *Lithobius forficatus*, was the only species recorded in the ungrazed grassland.

After the above examination, weeds were allowed to grow freely for

Group		1920 May	1920 July
Nematoda	Nematodes	—	4
	Long opaque forms	—	1
Oligochaeta	Small white forms	7	25
	Lumbricus terrestris	1	12
	Helodrilus oculatus	—	7
	Allolobophora chlorotica	—	1
Myriapoda	Symphyla	2	20
	Brachygeophilus truncorum	—	1
	Geophilus longicornis	2	2
	Lithobius forficatus	—	3
Acarina	Gamasus fimetorium	1	2
	Pergamasus crassipes	1	4
	P. runcatellus	—	2
	P. meridionalis	—	1
	Nymphs	1	—
	Archipteria redux	—	6
Collembola	Onychiurus ambulans	93	23
	O. armatus	76	26
	O. fimetarius	93	23
	Entomobrya muscorum	—	1
	Isotoma viridis	—	16
	Isotomurus palustris	—	9
	Beckia argentea	—	3
	Sminthurus viridis	—	1
Coleoptera	Amara communis	—	1 A
	Nebria brevicollis	—	3 L
	Xantholinus linearis	—	6 A
	Polystethus sp.	—	2 L
	Agriotes obscurus	3 L	1 L
	Weevil sp.	3 L	1 L
Diptera	Trichocera hiemalis	—	1 L
	Tipula sp.	1 L	—
	Psila rosea	—	1 L
Hymenoptera	Myrmica scabrinodis	19	—

two months, when a further sample was taken. By this date the surface was fairly closely covered by a mixture of the following plants:

A few *Poa* grasses, *Dactylis glomerata*, *Euphrasia officinalis*, *Lapsana communis*, *Hypochoeris radicata*, *Fumaria officinalis*, *Achillea millefolium*, *Anagallis arvensis*, *Plantago lanceolata*, *Trifolium repens*, *Bellis perennis*, *Viola tricolor*, *Geranium bisectum*, *Cerastium vulgaris*.

With the appearance of this covering of vegetation the majority of the fauna became intimately connected with the roots, *i.e.* the population became once again by far the greatest in the upper three inches, decreasing with depth. There was, moreover, a substantial increase in variety of species. So that in density, variety and vertical distribution there was some approach to the pasture land. The totals for the July sample are included in the above table.

Curiously, the *Collembola* showed a fall in total numbers, though the variety in species increased; the dominant form was *Onychiurus armatus*.

The *Achorutidae* generally were extremely small and Nematode attack was noted.

Among the other groups several species occurred which were not recorded for the ungrazed grassland or pasture, viz.: *Trichocera hiemalis* and *Psila rosea* among the *Diptera* and a *Scarabaeid* larva among the *Coleoptera*.

(3) CULTIVATED LAND. The land is about half the area of the pasture field and is situated immediately below the National Library on a steep slope about 4° from the horizontal (Fig. 1). To the N. other arable land adjoins it, while the remaining sides are bounded by more or less waste land. The position is sheltered to some extent from all but W.S.W. winds.

The primary purpose of obtaining samples from this area was for comparison with the pasture land. The seasonal cycle was however followed through six winter months.

Samples were taken from opposite ends of the area: (i) from a part with rows of *Trifolium*, (ii) from a portion with rows of *Perennial Rye grass*. Both these parts were kept carefully weeded.

Three years before the investigation, the ground was used for potato growing, being more heavily manured in the present clover section. The following year it was again manured and since then has not been touched. Early in 1920 the rye grass section was treated with a surface dressing of basic slag (10 cwts. to the acre). This difference in treatment has not, so far, led to any marked difference in the type of fauna occurring in the two parts, so that the organisms from these areas will not be discussed separately, with one notable exception in the group *Myriapoda*.

As was the case for the pasture area, the soil is open and porous in nature and the substratum shaley, so that there is no permanent water-logging; the slope of the land also tends to promote rapid drainage. The *water content* estimations showed no marked difference from the pasture field though they were often slightly higher—in one case as much as 7 per cent. This is possibly associated with the rather larger proportion of humus in the cultivated land. The figures for the weekly water content estimations of "representative" samples varied in a manner similar to those of the pasture area and are shown graphically in Fig. 6 in which is plotted also the census for the fauna of the arable land. The population curve of the pasture field falls lower and rises higher than that for this area. Fig. 7 shows the relative frequency of occurrence of the individual groups.

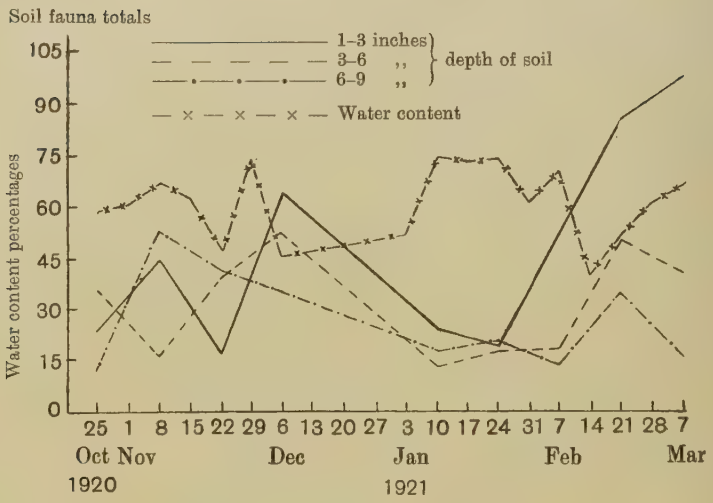


Fig. 6. Cultivated land: total fauna curves.

Distribution of the Soil Fauna of Arable Land*.

[2 samples per month]

Oct. 1920–Mar. 1921

Nematoda		
Nematoda spp.	A 2 / 10, 11	: B 1 / 11
Large white forms spp.	A 3 : 2 (1) / 11, 1	: B 3 / 10
Oligochaeta		
Small white forms spp.	A 20 : 5 (11) / 10, 11, 12, 1, 2	: B 52 : 8 (10) / 10, etc. to 3
Lumbricus terrestris	A 4 : 2 (3) / 2, 3	: B 1 / 12
Helodrilus oculatus	A 1 / 10	: —
Allolobophora chlorotica	A 1 / 1	: B 1 / 11
Arachnida		
(1) Gamasidae		
Pergamasus crassipes	A 5 : 2 (3) / 12, 1, 2, 3	: B 3 : 1 / 11, 1
P. septentrionalis	A 1 / 1	: B 3 / 11, 12, 2
Oligogamasus pollicipatus	A 1 / 11	: —
Gamasus fimetorium	—	: B 1 / 3
G. magnus	A 1 / 11	: —

[2 samples per month]

Oct. 1920–Mar. 1921

ARACHNIDA (*contd.*)		
Pachylaelops sp.	A 1 / 1	: —
Spp. Nymphs	A 2 / 12, 3	: B 1 / 11 —
(2) Thrombidiidae		
Bdella lignicola	A 2 / 11, 3	: —
(3) Oribatidae		
Archipteria redux	A 7 : 2 (1, 2, 3) / 12, 1, 2, 3	: —
MYRIAPODA		
(*a*) *Symphyla*		
Spp.	A 9 : 5 (11) / 10, 11, 12	: B 8 : 3 (10) / 10, 11, 2
(*b*) *Diplopoda*		
Polydesmus collaris	A 11 : 6 (11) / 11, 12, 1, 2	: B 30 : 8 (11) / 11, 12, 1, 2, 3
(*c*) *Chilopoda*		
Geophilus longicornis	A 4 / 11, 12, 1, 2	: B 13 : 6 (12) / 11, 12, 1
Schlendyla memorensis	A 2 / 10	: B 1 / 2
Brachygeophilus truncorum	A 2 / 1, 2	: B 3 : 2 (12) / 12, 1
Lithobius forficatus	A 6 : 4 (12) / 11, 12, 1	B 35 : 17 (11) / 11, 12, 1, 2, 3
INSECTA		
(*a*) *Collembola*		
(1) Achorutidae		
Onychiurus ambulans	A 133 : 73 (3) / 10, etc. to 3	: B 126 : 24 (3) / 10, etc. to 3
O. armatus	A 128 : 49 (2) / 10, etc. to 3	: B 87 : 24 (2) / 10, etc. to 3
O. fimetarius	A 24 : 10 (2) / 11, 12, 1, 2	: B 76 : 13 (11) / 10, etc. to 3
(2) Entomobryidae		
Entomobrya muscorum	A 6 : 3 (11) / 10, 11, 1	: B 3 : 2 (10) / 10, 2
E. multifasciata	A 14 / 11, 3	: B 19 : 17 (2) / 2, 3
E. Nicoleti	A 2 / 11	: —
Isotoma viridis	A 5 : 3 (2) / 10, 11, 2	: —
I. plumbea	A 1 / 2	: —
Isotomurus palustris	A 3 / 10	: B 1 / 11
Folsomia fimetaria	A 11 : 10 (2) / 2, 3	: B 19 : 10 (3) / 2, 3

[2 samples per month]
Oct. 1920–Mar. 1921

INSECTA (*contd.*)		
Beckia argentea	—	: $\frac{\text{B } 4:1}{11, 1}$
(3) Sminthuridae		
Sminthurus luteus	$\frac{\text{A } 1}{2}$	: —
(*b*) *Coleoptera*		
(1) Carabidae		
Nebria brevicollis (L)	$\frac{\text{A } 11:3\ (11, 12, 2)}{11, 12, 1, 2}$	: $\frac{\text{B } 5:1}{11, 12, 1}$
(2) Staphylinidae		
Xantholinus linearis (A)	—	: $\frac{\text{B } 2}{1, 2}$
Philonthus sp. (L)	—	: $\frac{\text{B } 3}{11, 1, 2}$
Sp.	—	: $\frac{\text{B } 1}{1}$
(3) Pselaphidae		
Euplectus kunzei (A)	—	: $\frac{\text{B } 1}{2}$
(4) Scarabaeidae		
Aphodius fimetarius (A)	$\frac{\text{A } 1}{12}$	: —
(5) Elateridae		
Agriotes obscurus (L)	$\frac{\text{A } 7}{12}$	: $\frac{\text{B } 2}{10, 12}$
(*c*) *Diptera*		
(1) Tipulidae		
Tipula paludosa (L)	—	: $\frac{\text{B } 1}{12}$
(2) Leptidae		
Leptis scolopacea	$\frac{\text{A } 1}{1}$	: —
L. tringaria (L)	—	: $\frac{\text{B } 1}{1}$
(3) Brachycera		
Sp. (L)	—	: $\frac{\text{B } 1}{11}$
(*d*) *Hymenoptera*		
Aphidius danci (C)	$\frac{\text{A } 1}{3}$	: —

* A formula similar to that previously used is employed above. The maximum number of individuals for any one sample is here given for B as well as for A, owing to the relatively greater concentration of numbers in the lower layers compared with that in pasture land.

The *maximum water content*, *pore space*, *hygroscopic water*, were all estimated from a similar number of samples to those used in the case of

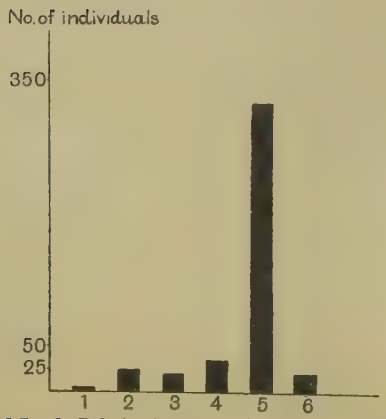


Fig. 7. *Cultivated Land.* Relative frequency of occurrence of individual groups.
Scale—1 cm. = 50 individuals.
1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera.
Totals of other groups too small to be included in diagram.

the pasture field. In each case the figures were rather higher for the arable land. The humus content was lower however.

	Average percentage in i. Arable land	ii. Pasture land
Maximum water content	84·25	81·56
Pore space	57·50	48·93
Hygroscopic water	3·70	3·45
Humus content	10·73	12·87

Whilst the temperature readings were generally similar to those of the pasture, the more sheltered position of the arable land was clearly shown by the records; in most cases the temperature of both the atmosphere and the soil was slightly higher than the corresponding readings for the pasture land.

Mechanical analysis of the soil of this area shows that it differs little from that of the pasture field in the majority of its components; the most notable points of difference are the greater percentage of coarse sand and the much reduced percentage of fine gravel. Stones of all sizes are much more numerous.

Standard samples of soil were taken every fortnight during the winter months and the faunistic results obtained (see tables) are collected in the previous table.

(i) As in the previous areas the forms present belonged to the groups *Nematoda*, *Oligochaeta*, *Myriapoda*, *Isopoda*, *Arachnida*, *Insecta*.

(ii) Both in number of species and of individuals all the groups which occurred are less than in the pasture area. In the pasture tables all the species of the arable land are included with the exception of the Chilopod *Lithobius forficatus*. The fact that the fauna occurred in such small numbers was probably due mainly to the lack of a turf covering and therefore of food, also to a lesser extent to the fairly frequent hoeing and weeding, resulting in a certain amount of direct injury also indirect through desiccation of the soil. These conditions were similar to those for the land recently ploughed.

(iii) The very small number of *Acarina* present may be particularly referred to.

(iv) The tables give the distribution in depth, the facts of which are shown clearly by the curves in Fig. 6. On comparison of the latter with the corresponding pasture land curves (Fig. 2), the outstanding feature is that the curves for the arable land show that the population of the upper three inches hardly exceeds that at a depth of from 3 to 6 inches or from 6 to 9 inches. A notable exception occurred on March 7th, where the totals for the upper and middle three inches had risen markedly above that for 6 to 9 inches which had fallen, but even here the middle curve closely follows the upper. In three samples the numbers which occurred in one or both of these lower layers even exceeded those in the first three inches. This is comparable with conditions in the recently ploughed area.

(v) Of the *Insecta* the *Collembola* were ubiquitous and the best represented in the area, though the variation in species was not so wide as in the pasture field; the same species were dominant in both, viz.: *Onychiurus ambulans* and *O. armatus* which together contributed 72 per cent., approximately, of the total Collembolan population. Young forms were found fairly evenly distributed throughout the samples, but all sufficiently developed for identification.

Cast skins occurred very frequently at all depths and in all samples.

The individuals throughout the samples were mainly from the *Achorutidae*. A greater proportion than usual of *Entomobryidae* occurred below three inches depth, probably due to the loose nature of the soil.

(vi) Among the *Oligochaeta* the small white forms alone occurred with any constancy; usually they were found coiled beneath small flat stones and fairly evenly distributed throughout the nine inches depth of the sample.

Points of lesser interest were:

(i) The occurrence of large numbers of *Myriapoda* in the samples taken November 22nd and December 6th. Individual plants of Perennial Rye grass had been planted a short time before, and a complaint made to the effect that the plants were dying off. On examination of a sample taken from the affected area on November 22nd, the trouble was found to be due to numbers of the small Diplopod *Polydesmus collaris*, of which 22 were found in one sample, rapidly eating away the roots of the plants in question. The Chilopod *Lithobius forficatus* was also present in similar numbers, a very active small carnivorous organism.

A fortnight later, the Rye plants were no longer dying off and the numbers of *Polydesmus* were found reduced to an average of ten per sample and *Lithobius* to seven. Besides the reduction in numbers of the injurious species, the plants had probably completed the critical unsettled period after planting out. *Polydesmus* did not occur before or after this particular period.

The *Symphyla* were less common than in the pasture field.

(ii) The Hymenopteran *Aphidus danci* occurred in the same unusual manner as in the pasture field, the pupa being free in the soil instead of living within a host.

5. ACKNOWLEDGEMENTS.

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6. NOTES ON PREVIOUS PAPERS AND SOME COMPARISONS.

The first intensive record concerning soil insects in relation to their environmental conditions, was published by Cameron (4). In that investigation and also in the present one, fields of permanent grassland were examined, though the Manchester area offered less uniform conditions affording several sub-habitats and included rotting trunks and vegetable refuse. Again the vegetation was much more heterogeneous than in Aberystwyth, in particular the variety of weeds. So that a qualitative comparison is hardly possible. A quantitative comparison is also impossible as Cameron did not work with standard samples.

The richer fauna in the Manchester field is probably due in part to the entering of species from the varied surroundings. The small number of *Collembola* found is probably due to the method of examining the soil; the smaller forms are difficult to distinguish unless the soil is sieved and the organisms floated upon the surface of water.

Cameron found forms occurring to a depth of 12 inches, while in Aberystwyth they rarely occurred below nine inches. In the case of the *Collembola*, however, while the Manchester forms were limited to the surface and first three inches, the Aberystwyth *Achorutidae*, at least, occurred frequently to a depth of nine inches or even below.

In both areas the roots *Poa pratensis* and *P. annua* formed food for similar Dipterous larval forms representing the families *Mycetophilidae*, *Bibionidae* and *Tipulidae*.

The second paper by Cameron(5) dealt with a similar investigation in Cheshire. It included many types of land from which "Glover's Meadow" is selected for a rough comparison; this is composed mainly of clay loam.

Only eight species are common both to this field and to the Aberystwyth area, out of approximately 58 and 85 species respectively.

A paper by Morris(19) forms an interesting and much closer comparison as he worked with a more limited area by means of standard nine-inch cube samples which is the size adopted by the present writer. Both areas have been in pasture for about 40 years, have a similar vegetation and are approximately the same height above sea level. Geologically, the Cheshire district presents a younger formation than Aberystwyth, though boulder clay overlies both. Mechanical analyses show very considerable differences between the types of soil; in Cheshire, the percentages of silt, coarse sand and fine sand being markedly greater and the other components considerably lower than those for Aberystwyth. In the Cheshire field a clay substratum is reached at about a foot deep and very little humus occurs below six inches, while at Aberystwyth the soil seems to be of the same nature for a considerable depth, probably until the shale rocks themselves are reached, and the organic content is as great or greater from six to nine inches as from one to six inches.

On consideration of the data recorded for both areas, the following points are evident:

(i) In both areas, the vast majority of organisms occur in the top two or three inches, particularly so in Cheshire.

(ii) That with the exception of the *Collembola* and *Diptera* there is

no great difference in the total numbers of species recorded in the two investigations. In the case of both these orders the number of species is more than twice as great in the Aberystwyth soil and the number of individuals is much greater.

(iii) That while the composition of the insect fauna of the soil in the two cases is mainly due to representatives of *Collembola*, *Coleoptera* and *Diptera*, the relative contribution of the orders expressed in species differs a good deal in the two cases. Of the total number of species represented by these three orders the *Collembola* contribute 30 per cent., the *Coleoptera* 46 per cent. and the *Diptera* 24 per cent. at Aberystwyth, the corresponding percentages for Cheshire being 19, 67 and 14. Few only of the species are common to the two investigations.

(iv) That while in each case a relatively small number of the species present provides the great majority of individuals, the dominant species in the two regions are not generally the same.

A second paper by Morris (20) deals with cultivated land and a comparison may be instituted with the cultivated land of the present paper.

The land of the two areas differs considerably in situation, nature of the soil and weeds—particularly the latter, which apparently are constantly present in the Rothamsted area, while the Aberystwyth cultivated land is kept carefully weeded.

In neither case did such a high percentage of the population occur in the first three inches as in grassland, but whereas Morris found the population of the first three inches markedly greater than the lower levels, in Aberystwyth it was only slightly in excess of the layers beneath. Perhaps this may be associated with the difference in quantity of weeds.

Morris deals in both manured and non-manured plots, and in both cases the variation in species is invariably richer than Aberystwyth. Very few of the species are common to both areas:

COLLEMBOLA: *Onychiurus fimetarius*, *O. ambulans*, *Isotoma viridis*, *Isotomurus palustris*, *Entomobrya multifasciata*.

MYRIAPODA: *Geophilus longicornis*.

ACARINA: *Gamasus magnus*, *Pergamasus crassipes*, *P. septentrionalis*.

A recently published paper by Buckle (3) concerns itself primarily with the effects of cultivation, but includes a Lancashire pasture field as a control. Comparing the latter with the Aberystwyth pasture one notes that the environmental conditions, geological formation of the land and the mechanical nature of the soil differ widely in the two areas. Buckle adopts the 9-inch cube as his standard sample.

Summarising the table for the "Big Meadow" and comparing this with the Aberystwyth data, one arrives at the table below, showing the number of species in the two cases (the figures in brackets indicate the number of individuals).

October to May inclusive.

	Lancashire pasture 1919–20 9″ cube of soil	Aberystwyth pasture 1920–1 9″ cube of soil	Aberystwyth pasture 1921–2 1–3″ depth
Acarina	—	15 (1149)	13 (2079)
Chilopoda	3 (3)	2 (13)	1 (4)
Diplopoda	—	1 (3)	1 (1)
Symphyla	—	? (71)	? (30)
Collembola	—	20 (1453)	18 (5292)
Rhynchota	—	2 (3)	2 (4)
Coleoptera	18 (29)	20 (95)	20 (115)
Lepidoptera	—	—	3 (3)
Diptera	6 (21)	16 (248)	12 (136)
Hymenoptera	1 (1)	1 (1)	—
Totals	28 (54)	77 (3036)	70 (7664)

Buckle's method of investigation involved careful sieving, but no floating out of small forms on water was undertaken. This difference in methods may explain the absence of *Collembola*, *Acarina* and *Symphyla* from Buckle's records and their presence in Aberystwyth samples, where the two former orders at least are the dominant forms.

In the same paper Buckle draws the following conclusions:

(i) That distribution and numbers are more stable in the fauna of grassland than of arable land.

(ii) That there is a seasonal increase in the fauna of *grassland* as the growth of vegetation increases.

(iii) That there is a seasonal increase in the fauna of *arable* land as the growth of vegetation increases.

The Aberystwyth data amply support No. (i) above, but more doubtfully (ii) and (iii).

The total fauna numbers increase markedly in early winter for Aberystwyth while Buckle says that his increase takes place with the growth of vegetation. But his orders only include *Chilopoda*, *Coleoptera*, *Diptera* and *Hymenoptera*, while the main orders determining the Aberystwyth curve are the *Collembola* and *Acarina*.

If analysis be restricted to the dominant groups of Buckle, viz. *Coleoptera* and *Diptera*, then as in Lancashire there is certainly an increase in Spring, but in addition there is another increase in early Winter.

However, it seems dangerous to generalise from such small totals, particularly as the inclusion of colonies has a wide influence on the trend of the total fauna curve.

The highest numbers for Aberystwyth in the following tables (136, 52, 91) are due to colonies of Dipterous larvae.

Number of Individuals per month.

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Aber. past., 1920–1	16	17	9	—	32	52	—	136	27	42	22	32
Aber. past., 1921–2	24	15	13	16	15	34	12	91	10	24	26	39
Aber. arable land, 1920–1 ...	—	—	—	—	3	7	15	9	6	0	—	—
Lancs. meadow, 1919–20 ...	—	—	—	—	0	0	0	4	11	7	17	11
Lancs. arable land, 1919–20	—	—	—	—	0	2	0	0	0	0	0	54
Lancs. "broken-up," 1919–20	—	—	—	—	5	0	0	1	3	1	11	31

7. SUMMARY.

1. An attempt has been made to give an account of a systematic qualitative and quantitative seasonal investigation of the macroscopic soil fauna of a limited area, and to correlate the data obtained with the general environmental conditions.

The piece of land most carefully investigated was a pasture field from which soil samples were obtained fortnightly throughout two successive years, but for purposes of comparison, observations were also made in connection with ungrazed grassland, similar ungrazed land with the turf recently removed and cultivated land; the soil of these areas is very similar in physical, chemical and geological characteristics.

2. The method of investigation included the taking of standard nine-inch cube samples. A preliminary sorting of the soil particles was followed by sieving the soil in water and placing the residue with water in a shallow dish, when the smaller organisms floated to the surface.

3. (*a*) The main area studied has been in pasture for over 40 years and has a typically mesophytic vegetation. Water content percentages were fairly high. The temperature of the soil varied with depth, being usually higher at 9 inches depth than at $4\frac{1}{2}$ inches; it varied much less than the temperature of the atmosphere.

(*b*) With rare exceptions the population in the surface three inches was markedly greater than that in the lower depths, probably because the majority of soil organisms are root feeders and the average root level ends at about three inches depth; carnivorous forms would naturally tend to concentrate here also. Another reason may be found in the better aeration of the soil in the surface layers.

(*c*) The orders *Collembola* and *Acarina* together determine the trend of the total fauna curve, *i.e.* they are the markedly dominant groups of the soil population as regards the number of individuals. A very few species are in turn responsible for the trend of the *Collembolan* and *Acarine* curve (Fig. 3); the high total numbers of the population are mainly contributed by the Collembolans, *Onychiurus ambulans*, *O. armatus* and to a lesser extent by *Isotoma viridis* and *Folsomia fimetaria*.

(*d*) Some orders such as *Collembola* and *Acarina* persist in varying numbers throughout the year, while other groups such as *Oligochaeta* and *Nematoda* entirely or almost entirely fall out for varying lengths of time. In the persistent groups, the same species remain dominant throughout the year.

(*e*) The maximum population occurs in the winter months; in part probably due to the relative absence of drought conditions in the winter, water content being more constant. The two summers have low totals for the fauna. Water content presumably plays an important part in connection with the small white *Oligochaeta*. Some preliminary experiments were carried out with regard to the effect of drought on *Collembola* and *Acarina*; it was found that life was very much curtailed by drought conditions.

4. The fauna of the *ungrazed grassland area* was similar to the pasture field except that the population was much greater. The richer character of the fauna is probably due to the protection of the long grass and the prevention of undue evaporation of moisture from the soil; being ungrazed the soil is less compressed.

5. The examination of *land recently ploughed* resolved itself into a survey of the results following (i) a removal of the turf layer; (ii) the further result of allowing the land so treated to be untouched for a definite time. With the removal of the turf, also goes the main source of food supply together with the majority of the soil fauna, and temporary conditions of drought are set up in the surface layers of soil. The main points of interest were: (i) the scarcity of the fauna except the *Collembola*; (ii) before weeds were allowed to grow the population of the upper three inches was *less* than that of lower levels, the numbers increasing with depth; (iii) with the growth of weeds the fauna showed some approach once more in density, variety and distribution to the grassland population.

6. The examination of *cultivated land* gave results very similar to those obtained for the previous area. The population is considerably less than that of the pasture. Generally speaking, the population of the

surface three inches of soil hardly exceeds that at a depth of three to six or six to nine inches. The same orders occurred as in the other areas.

7. Organisms injurious to the vegetation were present in all areas, but not usually in sufficient numbers to cause any damage. An exception occurred in cultivated land in which the roots of Perennial Rye grass were eaten away by the Diplopod *Polydesmus collaris*.

8. Parasitism was infrequent but at least the *Achorutidae* were subject to attack by small Nematodes.

Fungi were not normally found parasitic on the soil fauna; such attack appeared to be restricted to injured specimens.

9. A brief review has been given of work previously published and comparisons made with the present paper.

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THE LIFE-HISTORY AND HABITS OF *TORTRIX PRONUBANA*, HB., WITH SPECIAL REFERENCE TO THE LARVAL AND PUPAL STAGES

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(With Plate XV and 16 Text-figures.)

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Classification.

Order LEPIDOPTERA.

Group TORTRICINA.

Family *Tortricidae.*

I. INTRODUCTORY.

Tortrix pronubana is first recorded as occurring in Britain in October, 1905, when a single specimen of the moth was taken at Eastbourne (Adkin, 1905). It is recorded as having appeared in Guernsey in 1898 (Lowe, 1900). The moth is of Continental origin and was figured, without description, by Hübner in 1797 and 1830. According to Millière (1859), the species is a native of Southern Europe, Sicily, Corsica, Italy and Spain. Since its first appearance in Britain in 1905—it is evident that the moth has firmly established itself in the South of England—gradually working its way North. Within the past three or four years the species has become so abundant in the South as to prove harmful. In glass-houses at the Royal Botanic Gardens, Kew, serious damage is being done by the larvae to a great variety of plants.

The general life-history of the insect has been worked out by Adkin (1906 and 1908), who, however, has not given a detailed description of the larval stages. Accordingly, it was decided to conduct a further and more detailed investigation into its life-history and habits—with special reference to the larval and pupal stages. Material for the investigation was obtained from the Royal Botanic Gardens, Kew, and the insect reared in the laboratory on carnation plants and privet cuttings. Carnations have been badly attacked by this insect in France (Molinas, 1914). The laboratory experiments, conducted in the Entomological Laboratory of the Forestry Commission at Kew, under the supervision of Dr J. W. Munro, and also in Dr R. Stewart MacDougall's Laboratory in the University of Edinburgh, were amplified and supplemented by observations made in the glass-houses at Kew.

Tortrix pronubana is an interesting example of an insect which has within recent years appeared in our country from the South, gradually establishing itself and spreading northwards as it adapts itself to its new environment and climatic conditions. At the present time its actual distribution in Britain is not known but it would appear that, in the London district, where the work was carried out, the moth is present in large numbers, in the open, south of the Thames—while north of the river, although to be found, it does not occur abundantly. Continental

references to *T. pronubana* state that the larva is polyphagous. This is amply borne out under glass at Kew, but in the open the larvae are found abundantly, chiefly on privet, euonymus and ivy.

II. LIFE-HISTORY AND DESCRIPTION OF STAGES.

THE MOTH.

Description.

The following is a description of the moth given by Adkin (1907) and which appears to be the only one which has been published in Britain:

"*Tortrix pronubana* is easily recognised by the bright orange colour of its hind wings, which at once distinguishes it from all its allies that are known to occur in Britain.

"*The Male* measures from 15–17 mm. in expanse. Fore wings warm greyish brown, faintly reticulated with darker brown, with a broad rich red brown fascia from the middle of the costa, where it is narrowest, to the inner margin, where it broadens out, extends to the anal angle and unites with an irregular triangular patch of the same colour that occupies the apical and hind marginal areas. There is also a dark shade on the inner margin between the fascia and the base of the wing. The reticulations on the darker markings have a distinct bluish colour when seen in a bright light. Hind wings bright orange, bordered with black, with a few black scales scattered along the veins. Cilia of the fore wings, brownish orange, of the hind wings pale orange.

"*The Female* is a larger and more sombre insect. It measures from 18–22 mm. in expanse, is slightly paler in colour than the male and the reticulations are more clearly defined. The brown fascia is of a duller and less reddish tone and its central portion is often indicated only in outline as is also the triangular patch of the apical and hind marginal regions. The cilia of the front wings are brown from the apex for about two-thirds of their distance, where they shade into orange towards the anal angle. Those of the hind wings are orange. The orange rings of the body are more distinct than in the male. The female, when at rest, might easily be passed over as a pale example of *T. heparana*.

"There is little variation in the fore wings, beyond one individual being slightly darker or lighter than another, but the black scaling of the hind wings is subject to very considerable modification, chiefly in the males. In the paler examples, the black border is represented by a slender line, just within the fringes, and any further black scaling is hardly discernible. In other specimens the border is black and black scales are scattered

along the veins; while in the darkest individuals, the black scaling spreads over the wing so thickly as to almost obscure the orange colour."

Habits.

General observations on the life-history of the moth were begun in August, 1921, but no detailed studies were then made. Detailed observations were, however, commenced towards the end of May, 1922, pupae being collected from the glass-houses at Kew, the first moths emerging from these during the last week in May. The moths were then confined in cages containing young carnation plants. Food was provided in the form of sugar solution on a sponge and on blotting paper but the moths were never observed feeding. The duration of the life of the adult insect varies from 9 to 19 days, the sexes being approximately in equal proportion. Table I shows the proportion of the sexes to one another and the duration of adult life.

Table I.

Emergence 1922	Sex	Death 1922	Duration of adult life (days)
23. iii.	♀	3. iv.	12
6. v.	♀	19. v.	14
16. v.	♂	25. v.	10
24. v.	♀	9. vi.	16
25. v.	♂	29. v.	5*
,,	♀	5. vi.	11
,,	♂	3. vi.	10
26. v.	♀	5. vi.	10
27. v.	♂	9. vi.	14
,,	♂	,,	14
30. v.	♀	,,	10
21. vi.	♂	10. vii.	19

* Deformed specimen.

The moths are inactive during the day; it was usual for a moth to remain motionless in one spot all day long. In the greenhouses moths are rarely found during the day-time, unless disturbed from plants when they would fly a short distance and again settle down. In the early morning sun and towards dusk the adults become active and on summer evenings they were found in the open, in numbers flying about along with *T. unifasciana* in the neighbourhood of privet hedges where they had passed their larval and pupal stages. The male is much more active than the female—its flight being erratic and agitated; the female rarely flies, but when it does its flight is steady and it does not dart hither and thither as the male usually does. The moths do not

exhibit any marked attraction to artificial light—but in the laboratory it was found that they showed a tendency to keep at the side of the cage nearest the window.

Pairing.

So far as has been observed the female pairs only once in her lifetime, and that very shortly after the moths have emerged. In one case a newly-emerged male was put into a cage containing several females. It flew excitedly round the cage and paired immediately with one of the females. In this instance pairing lasted $1\frac{1}{2}$ hours.

Egg-Laying.

The female commences egg-laying almost immediately the act of pairing has been completed. In the laboratory eggs were laid occasionally during the day but much more frequently oviposition took place overnight or in the early morning and egg-masses were found in the morning which had not been present the previous evening. This confirms observations made in the open when the moths were found to be active in the early morning and in the evening. The eggs are laid in masses usually on the upper surface of the leaves of the host plant but occasionally masses are to be found on the under surface also. This position is, however, not common. In captivity in the laboratory, females have laid two or three batches of eggs on the under side of leaves of carnations and privet —in addition to several egg-masses on the upper surface.

THE EGG-MASS. (Fig. 1.)

The egg-mass consists of a number of eggs which overlap, resembling the shingles of a roof, and the shape of the mass, as a whole, apparently varies according to the shape of the leaf upon which it has been deposited. In the case of the long narrow leaf of the carnation the egg-mass is correspondingly long and narrow, while on the broad leaf surface of privet the mass assumes a distinct oval form. The eggs are laid in a number of masses which vary in size, the first laid masses being the larger, consisting in some cases of as many as 200 eggs, while the last deposited consist of only 10–20 eggs. The duration of egg-laying of a single female was found to be approximately 12–14 days during which 7 or 8 egg-masses were laid.

It is a common occurrence to find egg-masses deposited on the glass sides of breeding cages in the laboratory; on plant stakes and on the windows of green-houses. All fertile egg-masses laid on the leaves of host

plants in the laboratory hatched, as also did a large proportion of those laid on glass, but, frequently, these latter did not mature.

The flat egg-mass is at first light green in colour and is surrounded by a mucilaginous substance. Each egg, oval in shape, consists of a large mass of yolk surrounded by an outer wall which presents a regular reticulate appearance. The "cells" of the reticulations were found, on microscopic examination, to be generally 5-sided. Towards the edges of the egg they appeared more or less elongated. No micropyle is visible.

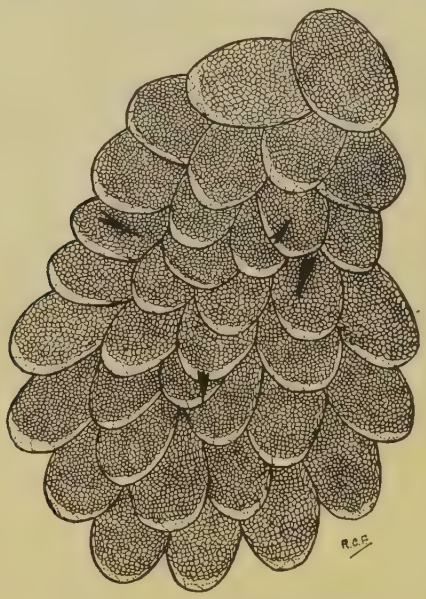


Fig. 1. Egg-mass of *T. pronubana* (magnified).

Incubation Period. (Table III, p. 402.)

The first egg-mass was laid in the laboratory on the leaves of carnations on May 27th, 1922, and egg-laying continued till the beginning of July. Conditions in the green-houses approximated to laboratory conditions but eggs had evidently been laid there prior to the end of May. When work was begun in May, 1922, larvae were present in the green-houses of a size which seemed to indicate that eggs had been laid in April. This was confirmed in spring, 1923, when it was observed that the first moths of the year commenced to emerge in the glass-houses from the end of March onwards.

The incubation period of the eggs varies from a minimum of 12 days to a maximum of 24 days, as far as observations have shown. The average period is 16–17 days. In 1922, eggs laid between 27th May and 10th June all hatched within a period of 12–13 days. The average temperature during this period was 69·6° F. On the other hand, eggs laid between 22nd June and 2nd July had an incubation period of 18–19 days. This longer period is explained by a fall in temperature. The average temperature during the period fell to 62·7° F. In May, 1923, the maximum incubation period obtained in the experiments (24 days) was accompanied by a still lower temperature—an average of 55·7° F. over the period. It is thus clearly shown that the incubation period of the eggs depends on temperature conditions. Moisture is, in all probability, another factor governing the development of the egg-masses. The death of many of the egg-masses laid on the glass sides of the breeding cages may be explained in this way.

Development.

The development of the eggs, as seen by the naked eye and low power hand lens, shows several successive changes in colour of the egg-mass. At first bright green, the mass becomes gradually darker green in colour and eventually each egg shows two small dark dots representing a pair of eyes. The egg-mass thus assumes a dark green colour—dotted all over with small dark brown points. The next stage of development is the appearance of the mandibles as dark objects and the egg-mass, as a whole, becomes greyish-green in colour. Just prior to hatching, the young larvae can be seen within the egg-shells actively opening and shutting their mandibles and twitching their abdomens. The larvae, on emerging, gnaw a hole in the egg-shells, push their heads through and thus rapidly escape. The first young larvae emerged in the laboratory on 7th June.

Table II.

The following table shows the number of egg-masses laid by two single females which had been fertilised and isolated.

Emerged	Pairing	Host	Oviposition and number of eggs per mass									Masses	Eggs
			1	2	3	4	5	6	7	8	9		
22nd June	22nd June	Carnation	22nd	23rd	25th	25th	26th	28th	5th	5th	6th	9	
			138	54	66	51	33	30	4	2	3		380–400
25th June	25th June	Privet	25th	26th	27th	28th	30th	2nd	2nd	3rd	6th	9–10	
			180 200	100	67	74	32	90	40	20	20		600–700

Table III.

Tabular Statement of Incubation Period of Eggs.

Host	Deposition	Hatching	Incubation period (days)
Carnation	28. v. 22	7–8. vi. 22	12
,,	,,	10. vi. 22	13
,,	30. v. 22	11–12. vi. 22	12–13
On glass of cage	,,	12. vi. 22	13
,, ,,	31. v. 22	11–12. vi. 22	12
Carnation	22. vi. 22	10. vii. 22	18–19
,,	25. vi. 22	12. vii. 22	18
,,	26. vi. 22	13. vii. 22	18
Privet	25. vi. 22	13. vii. 22	18
,,	28. vi. 22	15–16. vii. 22	18–19
,,	3. vii. 22	21. vii. 22	19–20
,,	30. iv. 23	20. v. 23	20
Carnation	3. v. 23	26. v. 23	23–24
,,	18. v. 23	7. vi. 23	21

Av. temp. 28th May–12th June, 1922 = 69·6° F.
,, 22nd June–7th July, 1922 = 62·7° F.
,, 30th April–7th June, 1923 = 55·7° F.

The Larva.

In the subsequent descriptions of the larval stages and duration of instars it is necessary to call attention to the fact that the observations were carried out during spring and summer, *i.e.* on the larvae of the summer brood. The duration of the instars, therefore, cannot be taken to refer to the larvae of the winter brood, where feeding and, consequently, growth is much slower and where the length of similar instars varies to even a greater extent than in larvae of the summer brood.

The arrangement of the setae or hairs which are found on the bodies of many lepidopterous larvae has been studied by Dyar (1901) and, within recent years, by Fracker (1915). In his *Classification of Lepidopterous Larvae*, the latter states that "the larvae of the family Tortricidae are of sufficient economic importance to warrant the expenditure of considerable time in the study of their structure." Accordingly, it was decided that it would be of importance to study the setal arrangement of the larva of *T. pronubana* on the lines of Fracker's work.

It was found that the 1st stage larva alone differs in its setal arrangement from the remaining stages—the newly-emerged larva lacking certain setae which appear at the first moult. Fig. 2 shows the distribution of the setae on the 1st stage larva while Fig. 7 shows the arrangement in the remaining stages (2–7).

Where differences occur in the external morphology of the larva at

its various stages, these are noted; otherwise, the detailed description of the full-grown larva (p. 408) also applies to the younger stages.

1st Stage Larva.

Length $1\frac{3}{4}$–2 mm. A series of measurements of the head capsule varied from ·23 ·26 mm. in breadth. The head is dark brown to black in colour. The thoracic shield is not prominent and is slightly darker than the rest of the body, being greyish-brown in colour. The remainder of the body is pale yellowish-green. The spiracles are elliptical with a dark margin. The prolegs are provided with uniordinal crotchets or hooks, *i.e.* all are more or less of one size. The crotchets number 9–10 per leg and are arranged in a circle which is complete on all legs with the exception of the anal proleg where the circle is not closed.

Arrangement of Setae. The larva is well clothed with stiff erect whitish bristles or setae and their distribution on the thoracic and abdominal segments is diagrammatically shown in the setal maps (Figs. 2 and 7). The maps have been prepared according to Fracker's method of representing the arrangement of setae. Each segment of the larva is shown on the map by a rectangle, the upper side of which corresponds to the mid-dorsal line and the lower side to the mid-ventral line.

The distribution of the setae of the 1st stage larva of *T. pronubana*—which, it will be recalled, measures only 2 mm. in length—cannot be observed without the aid of a binocular or a compound microscope. In studying this stage, in order to obtain a clear idea of the number and arrangement of the setae it was found necessary to use a compound microscope (Obj. $\frac{2}{3}''$) examining the larva, illuminated by artificial light and using a condenser. Studies of mounted specimens were also made.

The setae arise from slightly raised tubercles and in some cases several setae arise from the one tubercle—for example, the two setae immediately beneath the spiracle on the first eight abdominal segments both arise from the one tubercle. The tubercles are paler in colour than the surrounding epidermis, and, on microscopic examination, it is seen that they are devoid of the minute spines which are found all over the rest of the larval skin (Fig. 8). The setae are simple and arise from pits on the tubercles. They vary in length in different parts of the larva, the head carrying setae of the greatest difference in length.

Setae which are present in the 1st stage larva are termed by Fracker "primary": while those appearing at moult 1 are called "sub-primary." A comparison of the two setal maps (Figs. 2 and 7) shows that on the

prothoracic segment, the only difference between the first and subsequent stages is the absence in the former of one seta of the group A, which is found slightly below and anterior to the prothoracic spiracle. The same difference occurs on the remaining thoracic segments (cf. A 2, A 3—Fig. 7).

The setae on the abdominal segments are numbered after Dyar (1901). Seta V (Fig. 7) is not found on any segment in the 1st stage larva; it arises at moult 1 and is thus sub-primary. On segment 9, groups IV and VI have each one seta less in the 1st stage larva. The arrangement on segment 10 (Fig. 7) is similar in all stages.

Habits and Duration of Instar. On emerging, the young larvae are extremely active and immediately wander rapidly over the food plant. They are markedly positively phototrophic, always crawling towards the light. Never at any other stage in its development was the caterpillar so active—crawling rapidly about and, if in the least disturbed, dropping at once suspended by a long silken thread. In the laboratory the larvae were found to congregate at the tips of the leaves of the carnation plants, hanging in festoons on their threads and spinning quickly up and down. Adkin (1908) states that the newly-emerged larvae wander long distances from their birthplace before settling down to feed. Observations at Kew confirmed this, to a certain extent, but there was little difficulty in getting the young larvae to settle on the plants on which they were born. In the green-houses, on the other hand, it was quite evident that larvae wandered from plant to plant and were thus rapidly scattered, although all derived from one particular egg-mass. This wandering habit of the 1st stage larva undoubtedly, as Adkin has pointed out, gives the young caterpillar every opportunity of obtaining a plentiful food supply.

At this stage the larvae settle on the upper surface (occasionally on the lower surface) of the tips of leaves, in the leaf-axils and in the flowers of carnations—either singly or two or three together. After settling, a shelter of silk is soon spun and the caterpillar commences feeding upon the upper epidermis of the leaf. On other hosts—for example on those the leaves of which have a broad surface—it was a common occurrence to find two leaves spun together, the upper surface of one to the lower surface of the other and, between the leaf surfaces, two or three larvae feeding on the epidermis. Where larvae settle in flowers, *e.g.* carnation, the petals are spun together and perforated with several holes produced by the feeding of the caterpillars. Occasionally it was found that the 1st stage larva fed for a short time in one place and then wandered on to another. Usually, however, it remained in one spot till after the first moult.

All the larvae which emerged on 8th June and had been feeding in the laboratory on carnation plants moulted for the first time on 18th June. The duration of the 1st instar was therefore 10–11 days. This period was again observed in subsequent batches of larvae which were reared in the laboratory.

At moult 1—as also at all other moults with the exception of the last—the cast skin is eaten by the newly-moulted larva and only the cast head capsule is left to indicate the occurrence of a moult. This habit has been shown to be also true of the Holly Tortrix Moth (*Eudemis naevana*, Hb.) (Huie, 1917).

2nd Stage Larva.

Length 3–4 mm. Breadth of head ·30–·33 mm. This stage can readily be distinguished from the 1st stage larva by the colour of the head—which is distinctly light brown—as compared with the dark head of the previous stage. The thoracic shield is more distinct and there are now indications of dark markings appearing upon it. The colour of the body is of little value in distinguishing the stage. In general, the larva is rather more greenish in colour, as a result of feeding, but it was found that, as in all other stages, the colour varied with the food plant. In some cases the larva maintains the pale yellow-green colour of the first stage but in the laboratory, where larvae were reared on carnations, they showed a more pronounced green in their coloration. The crotchets of the prolegs are uniordinal—12–15 crotchets per leg.

Habits and Duration of Instar. The habits and method of feeding of this stage are similar to those of the 1st stage larva. After the first moult the caterpillars continue feeding in the same position as before, spinning a slightly longer and denser covering and, as a result of their increased size, eating more rapidly, but still feeding upon the epidermis of leaves or upon the petals of the flowers.

The duration of the 2nd instar was again found to be, on the whole, constant for larvae of the same brood. Larvae which moulted for the first time on 18th June moulted again on 27th–28th June. The duration of the 2nd instar is thus 10–11 days—the same as that of the 1st instar.

3rd Stage Larva.

Length 6–7 mm. Breadth of head ·42–·48 mm. The head is light brown in colour with varying darker markings, now more distinct. The thoracic shield is also light brown and has four dark markings towards its posterior margin. The hooks on the prolegs are uniordinal, 15-20 (approx.) per leg.

Habits and Duration of Instar. At the commencement of this stage the larvae continue feeding as before but towards the end of the instar and throughout the remaining instars, not only the epidermis, but the entire leaf is eaten through. As the larvae grow older and larger they feed under a more dense silken covering and are usually completely hidden from sight by the rolling of the leaf or leaves on which they are feeding. The larvae do not remain on one leaf throughout their life but, as food becomes scarce and exhausted, through the withering of attacked leaves, they move to fresh food and again spin themselves a new home and re-commence feeding. The 3rd stage larva feeds singly; it was unusual to find more than one on a leaf. As the larvae grow older they become less active. Their readiness to hang down on threads when disturbed, so noticeable in the first stage, is not nearly so marked in the remaining instars.

The duration of the 3rd and subsequent instars varies very considerably. Owing to the habit of the larvae of feeding completely hidden between spun together leaves and, further, as a result of their wandering about on the plant, it was found extremely difficult to follow the development of individuals. The duration periods, therefore, of this and the remaining instars, are probably only approximate. Larvae of one brood (*i.e.* emerging from the same batch of eggs) were, however, followed throughout their development and the results obtained give approximately the length of duration of the different stages of larval growth. In rearing a batch of larvae emerging at one time, as was done with *T. pronubana*, it was of interest to notice that during the first two larval stages the duration of the instars remained approximately the same for individual larvae. In the third and subsequent stages, however, some larvae grew much more slowly than others and, as a result, the duration of the instars varied to a great extent.

The duration of the 3rd instar varied from 9–20 days. The first 3rd stage larva moulted on July 6th—*i.e.* moult 3.

4th Stage Larva.

Length 8–10 mm. Breadth of head ·62–·67 mm. The head is light yellow-brown with various darker markings.

The thoracic shield is light brown with four dark patches on its posterior border, as in the 3rd stage larva.

In the laboratory, the larvae reared on carnations had now begun to assume a more pronounced olive-green colour. The tubercles from which the setae arise are more prominent.

The crotchets of the prolegs are still uniordinal and number approximately 25–30 per leg.

Duration of Instar. As in the 3rd instar, there is a great variation in the duration of the 4th instar. The first 4th stage larva moulted (moult 4) on 18th July, and cast head capsules of 4th stage larvae were found continuously in the laboratory experiments up till the middle of August. The minimum length of the instar was 8 days and the maximum observed was 20 days.

5th Stage Larva.

Length 10–12 mm. Breadth of head ·78–·85 mm. The markings on head and thoracic shield are similar to those found in the 4th stage larva.

The body is more distinctly olive-green in colour with tubercles rather more prominent than heretofore.

The prolegs now are provided with two types of crotchets—some hooks being longer and others shorter. As yet, no definite arrangement of the two types is found. The 5th larval stage therefore differs from the preceding four instars, in the structure of the prolegs. The crotchets again number approximately 25–30 per leg.

Duration of Instar. The duration of this instar again varies. Two larvae were found on 21st July which had apparently moulted for the fifth time. Their cast head capsules were not, however, found. This phenomenon was noticed on several occasions—6th stage—or apparently 6th stage larvae, being found but no cast heads were obtained to confirm the occurrence of the suspected moult.

In the laboratory moult 5 was recorded from 27th July and at intervals onwards from that date.

The minimum duration of the 5th instar was 9 days—the maximum was not determined.

6th Stage Larva.

Length 12–15 mm. Breadth of head ·93–1·1 mm. Head light yellow-brown with numerous dark patches as found in the 4th and 5th stage larvae. Thoracic shield yellow-green with sundry markings at its posterior margin.

The dorsal surface of the body is of a darker olive-green than the ventral surface—the anal segment on the dorsal surface is, however, also light green in colour. The tubercles are paler than the rest of the body and the setae long and distinct.

The crotchets of the prolegs are now distinctly biordinal and are

arranged so that long and short hooks alternate (Fig. 8). The crotchets number approximately 40 per leg.

Duration of Instar. The first 6th stage larva moulted on 3rd August. The duration of the instar again varied but observations showed a minimum of 10–11 days. In two recorded instances the duration was 17–18 days, but it was not possible to determine the maximum period.

7th Stage Larva. The full-grown Larva.

General Description. The full-grown larva (Fig. 3) measures from

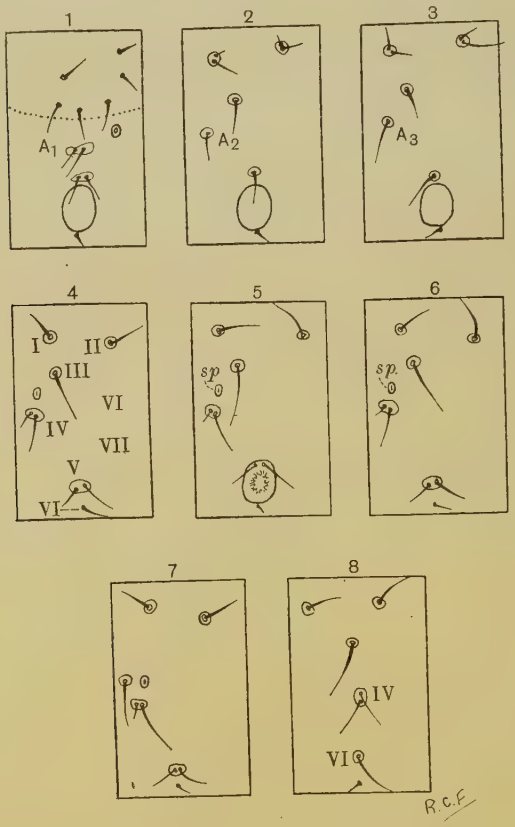


Fig. 2. Setal maps of 1st stage larva.

Map 1, Prothorax; 2, Mesothorax; 3, Metathorax; 4, Abdominal segments 1 and 2; 5, Abdominal segments 3, 4, 5, 6; 6, Abdominal segment 7; 7, Abdominal segment 8; 8, Abdominal segment 9. *Sp.* = Spiracle.

I, II, III, etc. = Names of groups of setae after Dyar (1901). A 1, A 2, A 3—compare with A 1, A 2, A 3, Fig. 7.

15–20 mm. in length and is cylindrical in shape, tapering slightly towards both ends. The head, which varies from 1·2–1·5 mm. in width, is of a glabrous yellow-brown colour with various dark markings, the size and position of which differ in individuals.

The thoracic shield is green—rather paler than the dorsal surface of the segments of the body—and is irregularly marked with dark brown patches, most typically four more or less triangular markings, along the posterior margin of the shield.

The dorsal surface of the mesothorax and metathorax and of abdominal segments 1–9 is olive-green in colour, while the ventral surface and the entire 10th segment are of a much paler green.

The spiracles are broadly elliptical in shape and have dark margins: the prothoracic and 8th abdominal spiracles are larger than the others and the 8th is slightly more dorsal in position. With the exception of the prothoracic spiracles, which are situated in the posterior half of the prothorax, all the spiracles are found towards the anterior boundary of their segments and are placed between two tubercles which bear setae. A single seta arises just dorsal to the spiracle and two, with their origin on one tubercle, just ventral and slightly anterior to it.

There are numerous folds in the body wall of the larva (Fig. 3). Each segment is divided dorsally by a transverse fold towards the centre of the segment, while a longitudinal fold runs just above the legs, throughout the entire length of the larva, with the exception of the prothoracic and 10th abdominal segments.

The larva is well clothed with stiff white hairs, the arrangement and distribution of which are discussed below (p. 415).

Detailed Description.

The Head (Figs. 4 and 5). The head is slightly smaller and narrower than the thoracic shield and can thus be partly withdrawn beneath the



Fig. 3. Full-grown larva of *T. pronubana*. ×5 (approx.).

shield. In a normal position the latter extends slightly over the occipital region of the head. It is not depressed and is glabrous with, as previously stated, sundry dark markings at its base.

The ocelli, which are situated on a dark patch, are 12 in number—six on each side of the head—immediately posterior to the antenna. The first five ocelli are arranged in a curve and are just visible dorsally while the sixth is more ventral in position.

The dorsal aspect of the head, including the arrangement of the hairs, is represented in Fig. 4.

The Antennae are 3-jointed. The basal membrane of soft chitin is well marked and gives the antenna the appearance of having four joints. The basal joint can be withdrawn within the basal membrane. The middle

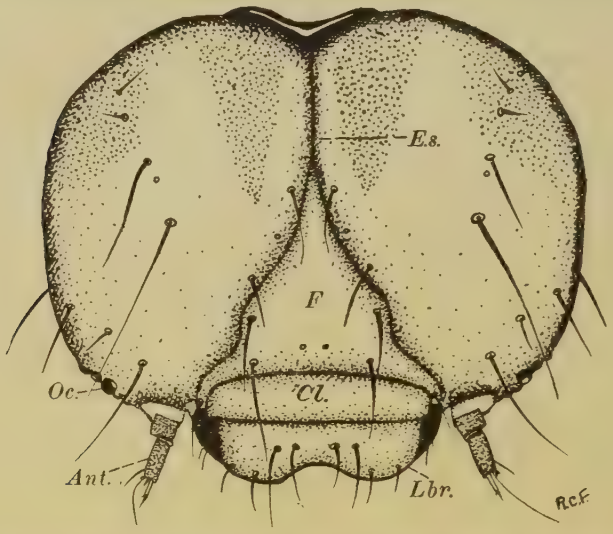


Fig. 4. Dorsal aspect of head of full-grown larva (magnified), showing distribution of hairs. *E.s.* = Epicranial suture; *F.* = Frons; *Cl.* = Clypeus; *Oc.* = Ocellus; *Lbr.* = Labrum; *Ant.* = Antenna.

joint is larger than the other two and is dark brown in colour. It carries two bristles, one, the longer, arising ventrally, and the other, the shorter, which arises from the dorsal surface. At the anterior end of this joint there are two small cones. The terminal joint is small and also carries at its apex a fine bristle and several small cones, one of which terminates in a fine hair.

The Labrum is twice as long as it is broad. The anterior margin, which projects inwards in the middle line, and divides the labrum anteriorly into two lobes, is more strongly chitinised than the remainder of the labrum. The "plate" of chitin thus formed is visible from the

ventral surface. Dorsally, on each side of the middle line, the labrum carries six bristles and their arrangement agrees with that figured by Trägårdh (1917) for the labrum of *Laspeyresia strobilella*. Bristles III and VI are marginal in position; IV and V are submarginal, while I and II are situated close to the middle line, some distance from the margin of the labrum and in line with bristle VI.

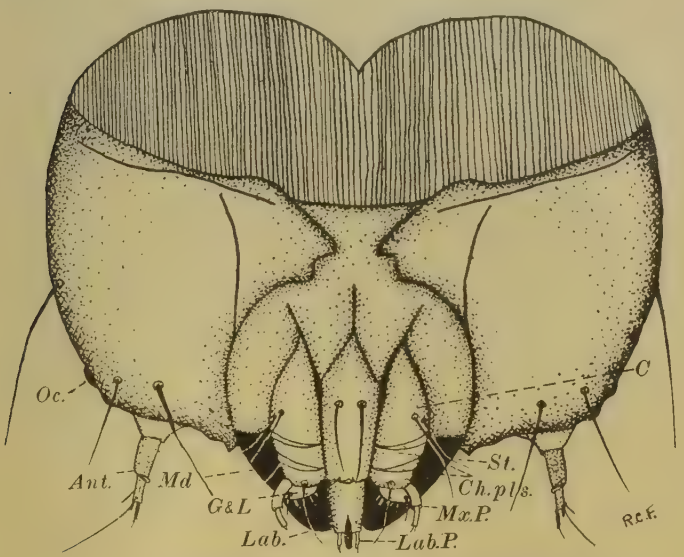


Fig. 5. Head of full-grown larva. Ventral aspect (magnified), showing mouth-parts and hairs.

C. = Cardo; *St.* = Stipes; *Ch.pls.* = Chitinous plates; *Ant.* = Antenna; *G. & L.* = Galea and Lacinia; *Mx.p.* = Maxillary palp; *Lab.* = Labium; *Lab.p.* = Labial palp; *Md.* = Mandible; *Oc.* = Ocellus.

Ventrally the labrum carries no bristles but is provided with six thorn-like chitinous projections, three on each lobe, towards its anterior margin. II is distinctly smaller than I and III, and in this apparently differs from Trägårdh's figure of the *Laspeyresia strobilella*. Further, the two chitinous plates which are also present towards the centre of the labrum of the above species, one on each side of the middle line, are in the labrum of *T. pronubana* entirely absent. The ventral aspect of the head and the mouth parts is shown in Fig. 5.

Mouth Parts (Fig. 5). In the ventral view the mandibles are seen lying behind the maxillae and the labium. The maxillae lie one on each side of the labium and each is connected to it by a strong chitinous rod. Each maxilla consists of the cardo and stipes, the latter being provided with two bow-shaped chitinous plates having the appearance of joints, but which, according to Börner (1907), are merely strengthening plates of chitin. Each plate bears a bristle at its anterior border, while some distance behind the posterior plate and on the border of the cardo and stipes, two other bristles arise of slightly differing length.

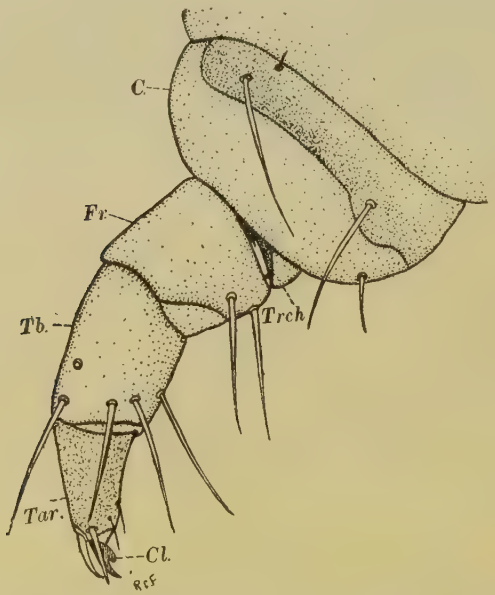


Fig. 6. Thoracic leg of full-grown larva. Posterior surface (magnified).
C. = Coxa; *Trch.* = Trochanter; *Fr.* = Femur; *Tb.* = Tibia; *Tar.* = Tarsus; *Cl.* = Claw.

The *Stipes* carries a two-jointed maxillary palp—the terminal joint of which bears several minute papillae and hairs. Also arising from the stipes and situated between the maxillary palp and the labium lie the fused galea and lacinia (or what apparently corresponds to the galea and lacinia). This structure carries six chitinous projections of varying size and shape. They are as follows: two small cone-like projections, each with two joints, the second of which is the smaller; two flat plate-like bristles, differing from ordinary setae in being broader and flatter

and not tapering gradually to a fine thread-like point; a minute bristle-bearing papilla and an ordinary seta arising close to the maxillary palp.

The *Labium* lies between the two maxillae and is modified, as in other

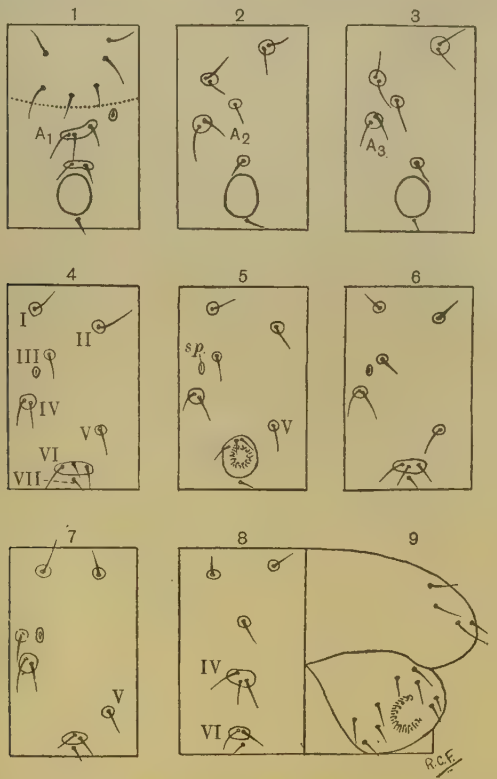


Fig. 7. Setal maps of larval stages, 2 and 7.

Map 1, Prothorax; 2, Mesothorax; 3, Metathorax; 4, Abdominal segments 1 and 2; 5, Abdominal segments 3, 4, 5, 6; 6, Abdominal segment 7; 7, Abdominal segment 8; 8, Abdominal segment 9; 9, Abdominal segment 10. *Sp.* = Spiracle.

I, II, III, etc. = Names of setae after Dyar (1901). *Note.* Presence of seta V—absent in 1st stage larva.

Lepidopterous larvae, to form the spinneret, at the tip of which the silk glands terminate. The labium is separated on each side from the maxillae by a stout rod of chitin. At the anterior end, on each side of the spinneret, is a small labial palp, two-jointed and terminating in a fine hair. The

labium carries two setae which arise slightly behind the posterior pair of bristles on the stipes.

Both in the dorsal and in the ventral aspects of the head, the mandibular condyle is visible as a small knob-like projection, situated between the mandibles and the antennae, but close to the former.

Thoracic Legs (Fig. 6). The thoracic legs are 5-jointed and consist of coxa, trochanter, femur, tibia and tarsus. Viewed from behind, the arrangement of setae and chitinous plates is as follows:

The basal third of the *coxa* consists of a chitinous plate at the posterior margin of which a minute bristle is situated. Two long setae arise from the middle of the plate one on either side. A fourth seta is situated immediately on the inner margin of the coxa.

The *trochanter* is a small joint and is provided with a chitinous plate and a clear area of articulatory chitin at its anterior margin. At each end of this clear area is a minute bristle.

The *femur* carries two bristles: one arises towards the inner margin and the other has its origin almost on the anterior surface of the joint.

The *tibia*, which is separated from the femur and tarsus by a clear area of soft chitin, bears four bristles or setae arising towards its anterior margin. A pit, indicating the possible origin of a fifth bristle, is seen towards the outer margin of the joint.

The *tarsus* is darker in colour than the other joints and terminates in a claw which consists of a sharp curved hook and a smaller blunt pad-shaped piece of chitin at the base of the larger claw and on its inner surface. Two short bristles arise from the inner margin, on the anterior third of the tarsus. In addition, the tarsus bears at its anterior end two thorn-like bristles, more plate-like than those arising from other parts of the leg. One of these bristles arises from the outer margin of the joint and the other over-lies the claw, with its insertion slightly posterior to the base of the claw.

On the anterior surface of the leg the joints are devoid of bristles, with the exception of the tibia which carries two setae towards its anterior margin and situated one on either side of the middle line of the joint.

Prolegs (Fig. 8). The prolegs of abdominal segments 3–6 are crowned with a complete circle of biordinal (alternating, longer and shorter) crotchets which number between 50 and 60 per leg. The anal prolegs do not have a complete circle—the crotchets extending only rather more than half-way round the foot. The crotchets are thickened plates of chitin which are bent at the apex in the form of a hook. For some distance

around the circlet of hooks, but on the leg itself, are numerous minute hair-bearing papillae.

A tubercle, bearing three setae, and which is free from any spines, is situated just above each proleg. A single seta, arising from a tubercle,

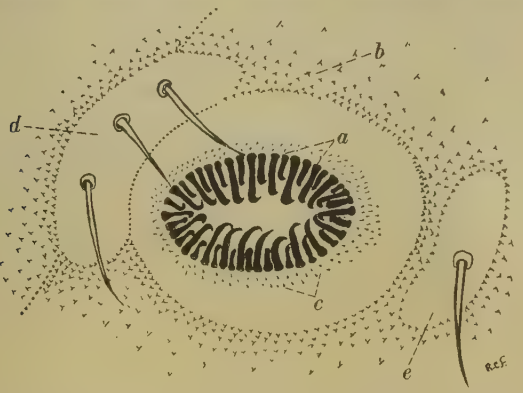


Fig. 8. Proleg (ventral view) of full-grown larva (highly magnified).

a, Biordinal crotchets; *b*, Small spines on skin of larva but which are absent on part of leg and on each tubercle from which setae arise; *c*, Hair-bearing papillae around base of crotchets; *d*, Group of 3 setae—Group VI; *e*, Single seta—Seta VII.

which is also free from any spines, is seen beneath each proleg and close to the mid-ventral line. The base of the proleg is devoid of the minute spines which cover the rest of the epidermis of the body of the larva.

Arrangement of Setae. Attention has already been drawn (p. 403) to the setal maps (Figs. 2 and 7) which have been prepared to illustrate diagrammatically the number and distribution of the setae in the 1st stage and subsequent stage larvae.

Adkin (1907–8) figures the dorsal view of a segment of the larva of *T. pronubana* showing the arrangement of the hairs. It is apparent, however, that his figure can represent the dorsal view of the first eight abdominal segments alone.

In his classification of Lepidopterous larvae, Fracker (1915) figures a segment of the larva of *Cydia pomonella* as representing the family Tortricidae. While the larva of *T. pronubana* agrees in most points with Fracker's conception of the arrangement in the Tortricidae, certain differences and variations occur which it may be of interest to note. For example, the arrangement on the 9th abdominal segment differs considerably from that on the corresponding segment of the larva of

C. pomonella. Further, in one case a variation was found in the number of setae composing Group VI (Fig. 7) on segment 8 of the abdomen. According to Fracker, the genus Tortrix, in common with several other stated genera, is characterised by this group being disetose. In one specimen of *T. pronubana* it was found that this group consisted of two setae on one side of the segment and of three setae on the other side. This non-symmetry was, however, only once observed. In all other respects the larva of *T. pronubana* was found to agree with the characters given by Fracker for the genus Tortrix. On the 8th abdominal segment, for example, the seta which, on the first seven abdominal segments arises dorsally and slightly posterior to the spiracle, on this segment invariably is anterior to and on the same level as the spiracle. Fracker believes that this is general in the Tortricidae except for a few rare cases.

Infra-Anal Comb. Situated beneath the anus, which is almost dorsal in its position at the apex of the 10th abdominal segment and posterior to the anal prolegs, on the ventral surface of the last segment there is

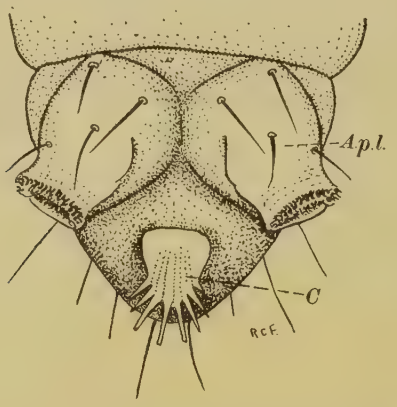


Fig. 9. 10th abdominal segment. Ventral view. Showing anal prolegs (magnified) and infra-anal comb.

A.p.l. = Anal proleg; *C.* = Infra-anal comb. (*Note*, 8-pointed.)

a chitinous flap which is prolonged into a number of sharp bristles. This flap or comb (Fig. 9) is present in all larval stages. The number of bristles varies from six to ten—the commonest number, however, being eight. In some cases the bristles are pointed but others are bifid or have a jagged end as though they had been broken. Examination of a number of combs never once showed one with a series of pointed bristles. It may

thus be assumed that the points have been broken either accidentally in making the microscopic preparations required for examination, or have been worn down through use. The bristles are arranged in a fan-like fashion according to their size. Where ten bristles are present the additional two are minute. In a dorsal view of the last segment of the larva, the apices of the bristles of the comb can be seen projecting beyond the posterior border of the segment.

The flap is hinged to the ventral surface of the body-wall and is capable of moving backwards and forwards through an angle of 180°. The apices of the flap can be brought together and closed in a fan-like manner. This flap appears to correspond with the "infra-anal lobe" described by Packard (1898) who states that his attention was first called to this lobe when examining some Geometrid larvae, where it takes the form of a thick conical fleshy lobe, ending in a hard chitinous point. He suggests that its function in the Geometridae is to aid in tossing the pellets of excrement away, so as to prevent contact with the body. It would appear that the hinged flap with its numerous hard chitinous bristles which is found in the larva of *T. pronubana* has a similar function. It is worthy of note that although the larva feeds beneath a silken web knitting closely together leaves or petals, its feeding ground was never found full of excrement; yet all around the twigs of privet and carnation plants, on which the larvae were feeding, in the laboratory, the excrement was plentiful. It is thus obvious that the pellets of excrement must have been ejected with some force from the home of the larva and tossed some distance away.

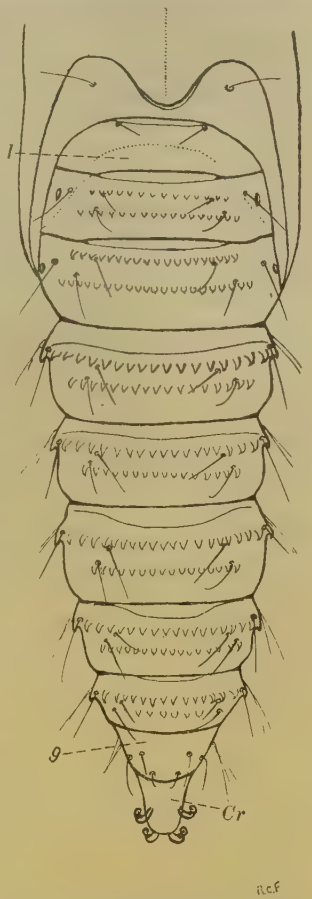


Fig. 10. Pupa of *T. pronubana*. Dorsal aspect of abdominal segments. × 12½. Showing arrangement of spines and hairs.

An anal-comb with eight points is found in the larva of *T. viridana* (Sich, 1915–6). It has, however, not been possible to ascertain whether such a comb is present in all members of the genus Tortrix or whether, indeed, it is to be found throughout the entire family Tortricidae. No mention is made of its presence in the larva of the Holly Tortrix Moth, *Eudemis naevana*.

Colour Variation in Larvae.

Reference has already been made to the variation in colour of the larvae which renders that character useless as a means of distinguishing the different larval stages. Observations made in the green-houses where larvae were feeding on a great variety of plants (see Appendix) showed that similar stages on the same plant varied to some extent in colour. Where a larva had been feeding on a yellow flower, *e.g. Cytisus fragrans*, its colour was very pale green with a more or less marked yellowish tinge. Such larvae transferred to the leaves of Cytisus gradually lost their yellowish tinge and became bright olive-green. Larvae on one plant in particular—*Nandina domestica*—were, with the exception of the 1st stage, always distinctly yellow in colour with a very slight trace of green. The larvae were feeding on the leaves of this plant in numbers: the plant was not in flower. Full-grown larvae reared on this host retained the yellow colour. A yellow dye is obtained from the wood of this and other plants belonging to the natural order Berberideae, an interesting fact which may be correlated with the yellow colour imparted to larvae feeding on its leaves.

Larvae reared on carnations in the laboratory were more or less of the same colour at corresponding stages but even in their case, also, there were differences in the shades.

It can be concluded that, as Poulton (1892, 1903) has shown from other Lepidoptera, the food plant has a distinct effect on the colour of the larvae feeding on it. At the same time, it was found that this effect was not the same on all individuals—larvae on the same hosts and at similar stages of growth, differing in colour from one another.

It was not, however, found that variations in colour of larvae resulted in marked variations of the adult moths. Pupae collected from many species of plants yielded adults differing slightly in density of coloration. In some specimens the markings on the fore wings were darker than in others. Adkin (1907) has drawn attention to considerable modification of the black scaling on the hind wings particularly in the male. It is probable that this slight variation in the adult may be correlated with

the colour variation in the larva but time has not permitted of a study of this matter.

Duration of 7th Instar. The duration of the instar, so far as has been observed, varies from a minimum of 14 days to a maximum which was not determined. The first pupa was obtained in the laboratory on 14th August. From that date onwards pupae were obtained from larvae which emerged on 8th June. In two or three cases pupation did not take place till spring, 1923.

THE PUPA.

Pupation.

Just prior to pupation the fully-grown larva completely covers itself with a sheath of silk, rather denser than that spun in the previous stages of its development. The leaves are thus tightly woven together and the pupa is invisible unless its shelter is torn apart and the mass of silk threads broken. Pupation takes place in a rolled leaf, in the axil of a leaf or between two or more leaves spun together.

Description and Chaetotaxy.

The pupa is at first brown in colour but later darkens, in most cases becoming quite black. In a few cases, however, the brown colour persists throughout the pupal period. The size varies from 9-12 mm., the average being 10 mm. As a general rule, the males are rather smaller than the females but this is not absolutely constant.

Dorsal Aspect (Fig. 10). The head, prothorax, mesothorax, metathorax, wings and nine abdominal segments and the cremaster are visible in a dorsal view of the pupa. The 10th abdominal segment is not visible. The 2nd and 3rd abdominal spiracles are visible and their position as well as the arrangement of the spines and hairs on the abdominal segments are shown in Fig. 10. The 1st abdominal segment does not bear any spines. Segments 2–8 inclusive, bear two rows of backwardly directed spines, one row near the anterior border of each segment and the other towards the centre. Segment 9 typically is without spines but in one case a pupa was found with the 9th abdominal segment bearing a central row of three or four spines (Fig. 11). The spines on all segments are extremely fine-pointed and, viewed in a certain light, appear as though they terminated in fine hairs.

The cremaster (Fig. 11) carries four pairs of hooks, two pairs lateral in position, which arise from the dorsal surface of the cremaster, and two pairs arising terminally from its ventral surface.

The dorsal surface of the pupa bears several yellowish hairs and the

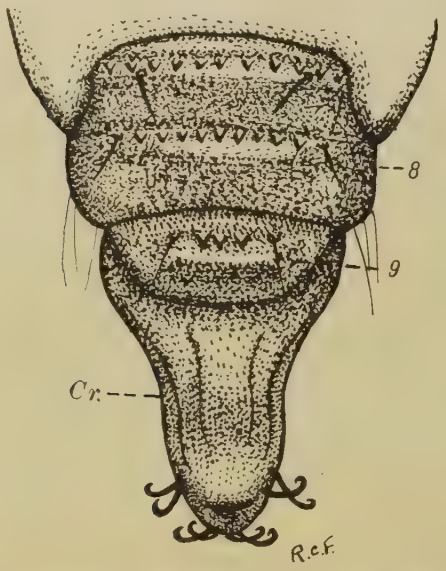


Fig. 11. Pupa of *T. pronubana*. Dorsal aspect of 8th and 9th abdominal segments and cremaster (highly magnified).

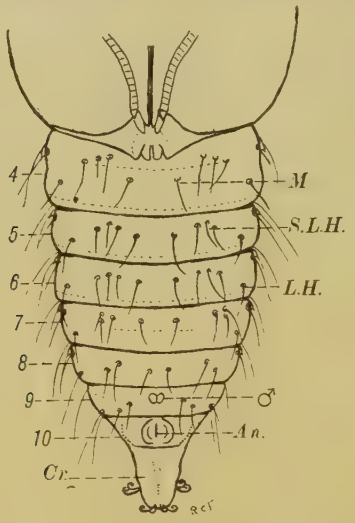


Fig. 12. Pupa. Ventral aspect of abdominal segments. $\times 12\frac{1}{2}$.
4–10=abdom. segments; *Cr.*=Cremaster; *An.*=Anus; ♂=male sex character; *M.*=Median Hairs; *S.L.H.*=Sub-lateral Hairs; *L.H.*=Lateral Hairs.

arrangement and distribution of these on the abdominal segments are also shown in Fig. 10. The hairs arise from pits and are not borne on tubercles as was found in the larva.

Ventral Aspect (Fig. 12). Abdominal segments 4–10 are visible from the ventral surface. The 10th segment carries the anus and is separated from the cremaster by an indistinct intersegmental line. The two pairs of terminal hooks on the cremaster arise from the ventral surface.

Numerous yellowish hairs are again found. Their arrangement is similar on segments 4–7, each segment bearing two single median hairs; two groups of three sub-lateral hairs, and two lateral hairs. The sub-lateral groups on segment 8 consist of two hairs only; while segment 9, although otherwise resembling the 8th segment, appears to lack the pair of median hairs. Segment 10 is without hairs and is indistinctly separated from the cremaster.

Lateral Aspect (Fig. 13). The first nine abdominal segments are easily distinguishable in a lateral view of the pupa. In the male chrysalis the 10th segment is not (in this view) visibly separated from the cremaster by one intersegmental line: in the female only an indistinct division is apparent. Thus, in neither sex, is the posterior boundary of the 10th segment well defined.

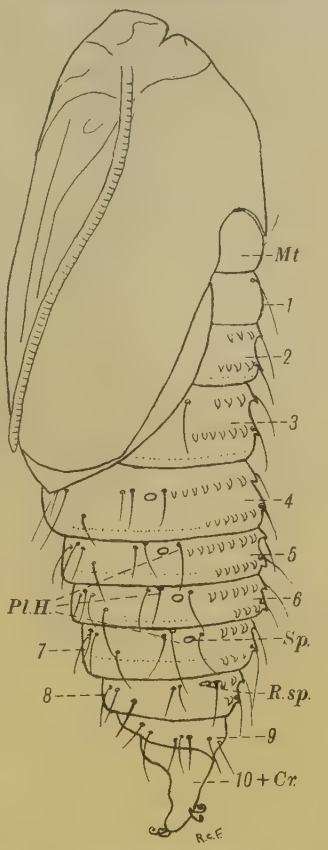


Fig. 13. Pupa. Lateral aspect. $\times 12\frac{1}{2}$.
Mt. = Metathorax; 1–10 = abdominal segments; *Pl.H.* = Pleural Hairs; *Sp.* = Spiracle; *R.sp.* = Rudimentary spiracle; *Cr.* = Cremaster.

Spiracles are visible on segments 4–7. The spiracles on the first three abdominal segments are hidden beneath the pupal wings. A rudimentary spiracle is present on the 8th segment. The arrangement of the pleural hairs is best described by reference to Fig. 13. In the lateral view of the pupa several of the dorsal and ventral hairs are also visible. The 10th segment and the cremaster do not carry any hairs.

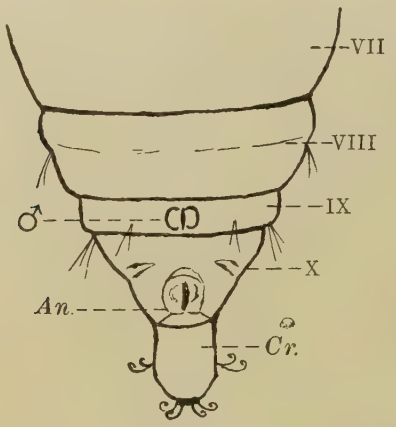


Fig. 14. Pupal sex character. Ventral surface of abdominal segments VII–X of male pupa of *T. pronubana*.

♂=sex character; *An.*=Anus; *Cr.*=Cremaster.

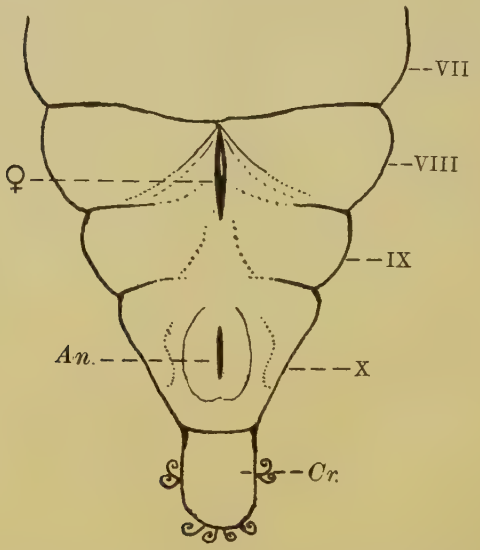


Fig. 15. Pupal sex character. Ventral surface of abdominal segments VII–X of female pupa of *T. pronubana*.

♀=sex character; *An.*=Anus; *Cr.*=Cremaster.

Pupal Sex Characters.

A knowledge of sexual differentiation in the pupae of *T. pronubana* has proved invaluable during biological work. The external generative organs of Lepidopterous pupae have been described by Hatchett-Jackson (1890) and Poulton (1890). According to both authorities the male sex of Heterocera is distinguished by a well-defined linear depression in the middle line of the ventral surface of the 9th abdominal segment. This depression is guarded by two trianguloid tubercles placed one on each side of the middle ventral line. According to Poulton the furrow between the tubercles is the ancient opening of the pupal vas deferens and now corresponds externally to the termination of the ducts internally.

Hatchett-Jackson summarises the characters of all the female Heterocerous pupae he has examined by "a triangular forward extension of the sternal region of the 9th abdominal segment, invading the sternal region of the 8th together with either (*a*) a linear depression on the 8th sternal region and another at the apex of the triangular extension, *i.e.* actually on the 9th segment, or (*b*) a single depression close to, or in the apex of, the triangular extension and produced by a confluence of the two depressions before mentioned." He further adds that, as a result of this forward extension of the 9th abdominal segment, the intersegmental line between segments 9 and 10 is not continuous from side to side across the ventral surface. Poulton's observations confirm those of Hatchett-Jackson and, at the same time, he discusses the actual segmental relationship of the two depressions referred to in (*a*) above. The anterior opening, according to Poulton, indicates the opening of the bursa copulatrix and is always associated with the 8th abdominal sternite. The posterior opening, on the other hand, which is for the oviducts, varies in its position and is often obscure and unrecognisable and is frequently fused with the anterior aperture. Poulton states that it may occur immediately anterior to the apex of the triangular projection of the 10th segment, *i.e.* on the 9th sternite, or on the projection itself and so on the 10th sternite.

Examination of pupae of *T. pronubana* has shown that as regards pupal sex characters the species agrees in general with those given by Poulton and Hatchett-Jackson and which are summarised above. The following notes on *T. pronubana* in particular may be of interest:

Male (Fig. 14). On the 9th abdominal sternite there are two knob-like tubercles lying between which, in the middle line, is a linear furrow or depression. The tubercles are easily visible by means of a hand lens.

The intersegmental lines between segments 9 and 10 are continuous from side to side.

Female (Fig. 15). The 9th segment extends forward in the form of a triangular projection invading the 8th segment. The apex of the extension reaches, in the middle line, to the posterior boundary of segment 7. In the middle line and on the extension of the 9th segment there is a slit-like linear depression extending the whole length of the extension and provided on both sides with prominent lips. Thus two separate generative openings are not visible in the pupa of *T. pronubana*: both are fused to form one long slit-like aperture. There is no pronounced forward extension of the 10th abdominal segment as Poulton figures for several Heterocerous pupae: in other words, the posterior boundary of segment 9 is not markedly extended forwards, the 9th segment projecting forwards distinctly from its anterior boundary. The intersegmental lines between segments 8 and 9, and again between segments 9 and 10, are not continuous across the ventral surface of the pupa.

Emergence.

Prior to the emergence of the moth the pupa wriggles itself out of its shelter by violently agitating its abdomen. In the pupa of *T. pronubana* abdominal segments 4, 5, 6 and 7 are movable in the male and 4, 5, and 6 in the female. According to Chapman (1893) this grouping of movable abdominal segments is found throughout the family Tortricidae. It is by the agitation of those segments and with the assistance of the backwardly directed spines on its dorsal surface that the pupa moves from its pupal shelter, remaining attached by the hooks on the cremaster to the silk which formerly had entirely surrounded it. The head and thoracic regions then split and the moth emerges, leaving the empty pupal case projecting from the leaves among which it had been protected. The moth, on first emerging, remains close to the pupal case till its wings expand. Pairing takes place soon after emergence.

Duration of the Pupal Stage.

The duration of the pupal stage was found to vary to a great extent. The minimum period recorded was 11 days while the maximum was 45 days. It was found that the pupal period of larvae of the winter brood was longer than that of larvae of the summer brood. Pupae kept under similar conditions—on the same plant and in the same greenhouse and which had commenced the pupal stage on the same day—opened after periods differing by as many as six days from one another.

Table IV shows the comparative duration of the pupal period of the summer and winter broods together with the variation that was observed in the case of pupae reared in Edinburgh and at Kew.

Table IV.

Pupation	Emergence	Duration of pupal period (days)
1921—*Summer brood* (Kew)		
7th Sept.	22nd Sept.	16
9th „	19th „	11
14th „	30th „	17
28th Aug.	9th „	13
1922—*Winter brood* (Edinburgh)		
17th March	16th April	31
23rd „	6th May	45
30th „	16th „	18
1923—*Winter brood* (Kew)		
18th April	16th May	28
23rd „	20th „	27
23rd „	18th „	26
25th „	17th „	22
8th May	7th June	30
16th „	11th „	26

Summary of Life-History.

There are two main broods of *T. pronubana* in the year—a summer brood which emerges from eggs laid in spring and a winter brood which emerges from eggs laid in autumn. During winter (October–March), 1921–2, larvae, which emerged in September at Kew, were transferred to Dr R. Stewart MacDougall's laboratory, Edinburgh, where the temperature corresponded to that at Kew (50–60° F.). The larvae continued to feed slowly throughout the winter months and showed no signs of hibernating. The first pupa was obtained from these larvae in March. The larval stage of the winter brood was thus prolonged to 5–7 months. The results of the observations in Edinburgh were confirmed in the laboratory and green-houses at Kew during winter, 1922–3. In one series of experiments where larvae were reared throughout the winter, under green-house conditions, the minimum period of larval life of the winter brood was 33 weeks.

The pupal period of the winter brood varies from $1\frac{1}{2}$–7 weeks (Table IV). Moths of the winter brood appear in April and continue to emerge throughout the spring.

The following notes on the summer brood are based on observations made at Kew. The first eggs of the summer brood are laid from the middle of April onwards. In the laboratory the minimum period of larval life of this brood was 68 days—10 weeks—and the duration of the pupal period averaged 15 days. Moths of the summer brood emerge from the beginning of August and onwards when they deposit the first eggs of the winter brood.

There is marked overlapping of broods. Larvae emerging from eggs laid late in spring do not mature in the autumn but feed along with young larvae of the winter brood derived from eggs laid in the autumn. Further, whereas it was found that the majority of larvae emerging in the beginning of June, pupated towards the end of August, a small number, on the other hand, continued in the larval stage throughout the winter and pupated the following spring. It is evident, therefore, that under glass, all stages of larvae can be found throughout the year.

Mr J. H. Durrant has informed the writer that moths were seen by him near London flying around privet hedges in the open during November, which further confirms the overlapping of summer and winter broods.

The observations and experiments conducted at Kew confirm those made by Adkin (1907). This worker also draws attention to the prolongation of the larval life of the species and the occurrence of "two fairly well defined broods in the year." It would appear that under glass, at the temperatures at which *T. pronubana* is found to be troublesome at Kew (50–60° F.), the two annual broods are also fairly well defined, the moths issuing in numbers in early spring and late summer. As in the open, the larval life is prolonged during the winter months.

Table V.

Stage	Autumn brood	Summer brood
Egg-masses	From Middle of August	April–June
Larvae	End of August–March, April	Majority feed April–August—a few over winter
Pupae	End of March, April and May	August onwards
Adults	Beginning of April onwards	Majority end of August to September. Others continue to emerge even into November

The following table (VI) gives a summary of the observations at Kew on the larvae and moths derived from single egg-masses of summer and winter broods respectively.

Table VI.

SUMMER BROOD.

Larvae reared on Carnations in laboratory.

Oviposition 27th–28th May.
Hatching 8th–9th June.
Pupation 1st pupa on 14th August.
Emergence 17 pupae all opened by beginning of October.
Duration of egg stage = 13 days.
,, larval ,, = 68 ,,
(,, pupal ,, = 15–17 days from 1921 observations.)
Minimum period egg-adult = 13–14 weeks.
Average temperature during period = 63° F.

WINTER BROOD.

Larvae reared on Carnation plants in green-house.

Oviposition —
Hatching 30th August.
Pupation *1st pupa 18th April—majority between 1st and 12th May.
Emergence 1st adult 16th May.
Duration of egg stage = —
,, larval ,, = 33 weeks (minimum)—average 35–36 weeks.
,, pupal ,, = 28 days (1st pupa)—average 27 days.
Minimum period from hatching of egg to adult = **37** weeks.

* Two larvae have still not pupated on 26th June.

Table VII gives a summary of the duration of larval instars of the summer brood. The larvae were all derived from a single egg-mass and were reared on carnations in the laboratory, the temperature of which corresponded to that of the carnation propagating house. Owing to the habit of the larvae in the later instars of wandering about on the host plants it was found impossible to follow individuals from stage to stage: as far as possible, however, this was done. As has previously been stated (p. 406) great variation occurred in the duration of the instars.

Table VII.

Emerged	Dates of moults (1922)							Lengths of instars in days						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
8. vi.	18. vi.	27-28. vi.	6. vii. 7. vii. and onwards	18. vii. 19. vii.	27. vii *30. vii.	*9. viii.	14. viii. 15. viii. and onwards							
				26. vii. and onwards	*1. viii. and onwards	*14. viii. 22. viii.	during Sept.	10–11	10–11	9–12 and over	8–20 and over	9–10 and over	10–26	14–22

Average minimum duration of instar = 9–10 days.
Average day temperature during larval life = 63·5° F.

* Denotes same larva followed throughout both instars.

III. ECONOMIC IMPORTANCE.

Host Plants.

The first recorded host plant of *T. pronubana* in the open in Britain is a species of Euonymus (Adkin, 1905). The species is now found in abundance in certain localities in the South of England feeding on euonymus and privet. At the Royal Botanic Gardens, Kew, it is present in large numbers, also on ivy and on laurel. It is only within the last three or four years, however, that the moth has made its appearance under glass at Kew and there the larvae are found feeding on all kinds of plants at temperatures varying between 50 and 60° F. A list of the plants on which the larva is found under glass is given in the Appendix, and, although not exhaustive, it illustrates the polyphagous habit of the species and shows the wide range of plants, embracing many Natural Orders, on which the larva thrives.

Through the kindness of the director, Mr Chittenden, a visit was paid to the Royal Horticultural Society's gardens at Wisley in August, 1922, and again in August, 1923. Mr G. Fox-Wilson the entomologist stated that he had never been troubled with *T. pronubana*. On both occasions a careful examination of the carnations and other plants under glass was made and it is interesting to note that no trace of *T. pronubana* was found. It is curious that an insect, which has proved so troublesome at Kew, should be entirely absent from Wisley and it is probable that the species occurs in abundance only in certain localities in the South of England.

A thorough examination of the glass-houses at the Royal Botanic Gardens, Edinburgh and at the Botanic Gardens, Glasgow showed that *T. pronubana* did not occur in either locality.

In Edinburgh, however, it may be noted that another Tortrix species was found in large numbers on a specimen of *Araukaria braziliensis* in the Large Palm House. The tree was badly attacked—the larvae not only spinning the leaves together and feeding on them but also boring into the stem and shoots causing exudation of resin, defoliation and death of leaves and shoots. Numbers of larvae were collected and reared in the laboratory at Edinburgh. Moths were obtained from these in the beginning of May and were identified by Mr Durrant of the British Museum as *Batodes angustiorana*, Haw.

INJURY TO PLANTS BY *T. PRONUBANA*.

Signs of Attack.

The first indications of *T. pronubana* attacks are afforded by the spinning habit of the species so characteristic of the family Tortricidae. Immediately after emerging the young larvae wander about in search of a suitable place to commence feeding and having found such a place they at once begin to spin a shelter for themselves. Where the plants attacked possess small leaves, *e.g. Cytisus fragrans* and *Pimelea* sp. the presence of the young larvae is more noticeable than in plants possessing larger and coarser leaves, *e.g. Dianthus* spp. In the former case several leaves are soon spun together while in the latter type of plant the spinning of the young larvae is not easily seen since they usually spin at the tips of leaves or in leaf axils. Consequently, the characteristic knots of twisted spun-together leaves, so obvious in small leaved plants, are not at first present. As the larva grows, however, they become more apparent.

Of the various host plants cited in the Appendix, *Dianthus* spp., *Cytisus fragrans*, *Acacia* spp. (in particular *A. longifolia* var. *mucronata*) and *Chorizema ilicifolium* are the most severely attacked. In all cases it was found that the larva feeds on the leaves and foliage, particularly at the young growing tips of the plants attacked. On carnations the presence of young stage larvae is difficult to detect.

In addition to feeding on the leaves it is common to find larvae attacking unopened buds. This is particularly noticeable in carnations and *Pimelea* sp. A small hole (Fig. 16) in the side of the bud is the only indication of infestation, the bud in all other respects appearing quite normal. Within such a bud, however, a larva is present, feeding on the flower within and thus entirely destroying it. In late autumn, November, the flower buds of *Pimelea* sp. were badly attacked.



Fig. 16. Carnation bud (enlarged) containing larva of *T. pronubana*.

Note hole, the only sign of attack. This is the typical appearance of attacked buds of different species of plants.

The larva does not confine its feeding to leaves and unopened buds

but also feeds on flowers, *e.g.* flowers of Carnations, Cytisus and Acacias are frequently found with larvae feeding upon them. A carnation flower attacked by 1st and 2nd stage larvae has its petals perforated with small holes resulting from the feeding of the young caterpillars.

Injury Resulting from Attacks.

The damage done to the host plants is due to the destruction of the leaf tissue and consequent reduction of leaf area. In the laboratory where young carnations were used for rearing the larvae it was found that a perfectly healthy plant was killed 6–8 weeks after infection with a number of larvae. The photographs (Plate XV) show three plants, before, during, and after an attack by *T. pronubana*.

In the glass-houses at Kew, however, the larvae are not present in sufficient numbers to cause the death of the plants; if, on the other hand, they were allowed to increase in numbers, there would be a grave risk of causing the death of the smaller plants attacked. Buds and flowers are alike destroyed and thus again injury of a different nature done to the plant.

Quite apart from the damage done to the health of the host plant, the presence of *T. pronubana* larvae tends to mar its beauty, causing bunches of twisted, shrivelled and withering leaves to appear (Plate XV, plant B), and flowers to become distorted and unsightly with the petals eaten and spun together. Such harmful effects are of considerable importance anywhere, and particularly in such places as the Royal Botanic Gardens, Kew, where it is essential to maintain the beauty as well as the health of the plants.

Mode of Dispersal.

Reference has already been made (p. 404) to the habit of the 1st stage larvae immediately after emerging of wandering some distance before settling down to feed. In a green-house, therefore, it is obvious that infection from plant to plant at this stage is a simple matter, especially when the polyphagous habit of the larva is remembered. Further, the characteristic habit of the young larva of hanging in festoons from the leaf tips is of importance with regard to the spread of the pest from one plant to another. The slightest draught waves these festoons to and fro so that they often come in contact with neighbouring plants. In a green-house there is not the same possibility of wind playing such a large part in the distribution of young larvae as in the open. It is frequent, however, to find quite a distinct draught in a green-house, especially on a hot summer's day when the "lights" are open.

The adult moths flying throughout the green-house obviously play an important part in dispersal since the females lay their eggs on practically any plant and also on the glass sides and roof of the house. It is apparent that the pest could be eradicated from any given glass-house if means were taken to prevent the entrance of moths from the open. The use of insect-proof screens of wire gauze, or, less expensive, of muslin which could be fitted to cover all openings when windows are open, would effectively prevent moths entering or leaving a house. In this way, each house could be separately treated for the pest and eventually it would be entirely eradicated. The screens, however, would require to be used permanently since the species breeds freely in the open on privet, euonymus and ivy.

A further means of dispersal is found in the introduction of infected plants into houses formerly quite "clean." In Botanic gardens it is the usual practice to transfer plants from propagating houses to the larger green-houses. Thus it is obvious that infected plants may be conveyed from house to house and ideal conditions provided for the spread of the pest. This means of spread could be remedied by having all plants carefully examined before transferring them from house to house.

IV. CONTROL.

The following series of experiments was conducted with a view to ascertain the most practical and effective methods of controlling *T. pronubana* under green-house conditions. A study of the life-history has shown that such experiments should be devised to deal with the young larvae immediately after emergence.

SPRAYING EXPERIMENTS.

A. *Effect of a Nicotine Spray on Newly-Emerged Larvae.*

Exp. 1.

Nicotine spray—Commercial XL–All: used in the proportion of 1 part to 30 parts water.

May 23rd. Plants sprayed; larvae on plants 1st stage—48 hours old.

May 25th. Plants examined—results as shown in Table VIII.

Table VIII.

Plant	Larvae dead	Larvae alive	Totals	% killed
A. (Carnation)	34	12	46	73·9
B. („)	14	7	21	66·6
C. („) (Control, unsprayed)	—	7 (All found)	7	—

With a few exceptions the larvae not killed were in places which the spray had not reached, *i.e.* protected in the axils of leaves and in several instances sufficiently protected from the spray by a silken shelter already partly spun.

Where the spray reached the larvae death resulted, but it is apparent that it is not possible to reach all larvae even 48 hours after emergence.

Exp. 2.

According to Gibson and Ross (1922) the following nicotine and sulphur spray was used with excellent results in green-houses in Eastern Canada against the Green-house Leaf-Tyer:

Phlyctaenia ferrugalis, Hb.

Soluble sulphur	1 ounce by weight.
Nicotine sulphate (40 %)	1 fluid ounce.
Water	6 gallons.

It was decided to use this spray in the above proportions on an experimental scale against 2nd and 3rd stage larvae of *T. pronubana*. The following are the results obtained:

July 6th. Three carnation plants A, B, C on which 1st, 2nd and 3rd stage larvae were present, since emergence, all thoroughly sprayed, as above.

July 7th. Plants examined.

A.	Larvae dead = 6	Larvae alive = 36
B.	,, = 2	,, = 17
C.	,, = 2	,, = 10
	Totals 10	63

Percentage killed = 16.

In all cases the larvae killed were 1st stage and so not protected to the same extent by spun-together leaves as the older larvae. It is evident that the small percentage destroyed is due to the fact that the spray did not reach the older larvae.

B. *Effect of Nicotine Sprays on Egg-Masses.*

Exp. 3.

Plants—carnations—each with an egg-mass on their leaves thoroughly sprayed with XL–All (1 in 30): egg-masses in all cases just about to hatch.

Table IX.

Plant	Sprayed	Larvae emerged	Remarks
A.	25th June	—	Egg-mass did not hatch: eggs shrivelled up
B.	28th June	30th June	All eggs hatched: but two dead larvae found beside empty egg-shells
C. Control and unsprayed		26th June	All eggs hatched
Four isolated egg-masses	30th June	First mass hatched July 3rd	By July 9th majority of eggs in sprayed and unsprayed masses had hatched: out of all larvae four or five died on emerging: % dead, negligible
Four isolated egg-masses	Control and unsprayed		

Exp. 4.

Egg-masses sprayed with nicotine and sulphur spray (see above).

June 25th. Mass A. Of approximately 60 eggs, sprayed.

,, B. ,, 40 ,,

,, C. Control and unsprayed.

June 26th. Mass C. All eggs hatched.

June 27th. Mass A. Majority of eggs hatched; 1 dead larva beside mass.

Mass B. Majority of eggs hatched.

June 28th. Mass A. All eggs hatched except four which were not yet mature.

Mass B. Two dead larvae beside mass; several eggs not yet hatched.

July 2nd. Remaining eggs hatched in both masses.

In the above experiments nicotine and nicotine and sulphur sprays in the strengths used did not prevent the hatching of the egg-masses. In one case only (Exp. 3, Mass A) did a sprayed egg-mass not hatch. It is likely that the death of this mass was due to some other cause since all other masses sprayed under the same conditions hatched.

The larvae, on emerging, settled down to feed, as usual, on the leaves of the plants, the nicotine by this time having apparently no effect on the young caterpillars.

In no case did the plants used in the above experiments with nicotine sprays suffer in the least.

C. *Effect of Arsenate of Lead Spray on Larvae.*

In the following experiments Strawson-Swift Lead Arsenate Paste was used in the proportion of 6 lbs. to 50 gallons of water. This strength of spray is recommended by Lloyd (1920) for the control of *Hadena oleracea*.

Exp. 5.

June 6th. Plant A. With an egg-mass of approximately 50 eggs just about to hatch; thoroughly sprayed as above.

June 8th. Egg-mass found hatched.

June 9th. Eight larvae found; many others wandering over plant and beginning to feed.

June 11th. Seventeen dead larvae; still a few feeding.

June 13th. Two dead larvae; no others visible.

Total larvae killed = 19 and plant clean.

In the control plant all leading shoots infected with larvae from an egg-mass which hatched on June 7th.

June 8th. Plant B. Infected with 1st stage larvae and then sprayed as above.

Plant C. Sprayed; eggs about to hatch.

June 9th. B. 1 dead larva.
C. Egg-mass just hatched.

June 11th. B. 1 dead larva.
C. 2 dead larvae.

June 13th. B. 2 ,,
C. 8 ,,

Both plants sprayed again with freshly-made spray: suspected that first spray on June 8th was not satisfactorily mixed.

June 15th. B. 10 dead larvae; several living larvae feeding on under side of leaves where spray had not reached.
C. 20 dead larvae.

June 18th. B. 6 ,,
C. 8 ,,

June 21st. B. 1 dead larva.
C. 1 ,,

June 25th. B. 2 dead larvae; none alive.
C. 0 dead larva; 1 alive.

Experiment closed.

Total larvae killed	B.	23
,, ,,	C.	39
Total		62

Although it is not possible in this experiment to estimate the actual percentage of larvae destroyed since many wandered away from the plants when they first emerged, yet it is apparent that 18 days after the hatching of the egg-masses the plants were found to be free from larvae.

The control plant at the end of the same period was showing signs of severe attack, the leaves being spun together and the tips of many withering where the young larvae had been feeding.

Effect of Lead Arsenate on Plants.

Carnation plants were used in the above experiments and they did not suffer in the least from the spray: the only effect of spraying being the pale whitish colour imparted to the leaves by the deposit of lead arsenate left as the spray dried.

FUMIGATION WITH HYDROCYANIC ACID GAS.

With a view to ascertaining whether hydrocyanic acid gas was of any value for destroying the larvae of *T. pronubana* a series of experiments were conducted in a small green-house (cubic contents 828 cu. ft.) specially prepared for the purpose. The following is a summary of the experiments conducted. Valuable information regarding fumigation with hydrocyanic acid gas and the quantities recommended were obtained from Bulletin No. 513 (Sasscer and Borden, 1917) of the U.S. Department of Agriculture.

Exp. 1. 14th August.

Strength of cyanide used = $\frac{1}{4}$ oz. per 1000 cu. ft.
Exposure = $1\frac{1}{4}$ hours.
Temperature during exp. = 62° F.

Table X.

Table of Results.

Plant	Alive—before exposure	Dead after exposure	Remarks
Carnation	12 (3rd–7th stages)	0	Only one carnation plant showed any sign of burning of leaves
„	7 „	0	
„	4 „	0	
„	3 „	0	
„	2 „	0	
Coleonema albuma	4 (2nd–4th stages)	0	Slight burning of leaves
Rhododendron Edgeworthii	1 (7th stage)	0	
Lhotskya ericoides	1 „ „	0	
Acacia Drummondii	4 (3rd „ 14th „)	2*	This plant was nearest jar containing cyanide
Acacia pulchella	5 various stages	1*	
	% of larvae killed = 7.		

* In the light of subsequent experiments, with stronger doses of cyanide, it is doubtful whether the death of these larvae resulted from hydrocyanic acid gas.

Exp. 2. 15th August.

Plants as in Exp. 1 with the surviving larvae.

Strength of cyanide = ½ oz. per 1000 cu. ft.
Exposure = 2 hours.
Temperature = 67° F.
Percentage of larvae killed = nil.

Exp. 3. 25th August.

Plants and larvae as in previous experiment.

Strength of cyanide = 1 oz. per 1000 cu. ft. (requisite quantity divided between two jars so as to ensure better distribution of gas).

Exposure = 2½ hours.
Percentage of larvae killed = nil.

Exp. 4. 26th August.

Plants and larvae as before.

Strength of cyanide = ½ oz. per 1000 cu. ft. but in two jars.
Exposure = 12½ hours.
Percentage of larvae killed = nil.

During the above experiments larvae were also placed in the green-house in petrie dishes covered with muslin and thus exposed to the gas. In no case, however, were larvae killed.

It may be concluded that fumigation of plants infected with larvae of *T. pronubana* is of no value as a control measure.

Further Experiments.

Pyrethrum powder burnt in a large cage in which had been placed a plant attacked by larvae of *T. pronubana* at various stages was found to have no effect whatever on the caterpillars.

Fumigation with tobacco fumes as is regularly done at Kew to destroy green-fly had also no effect on the larvae: nor were moths destroyed which had been confined in a cage—two sides of which were made of wire gauze—the cage being placed in a green-house during fumigation with tobacco.

Conclusions drawn from Control Experiments.

The control of *T. pronubana* is a difficult problem and the brief experiments cited above are sufficient to show that it is impossible to outline complete and successful control of the insect without a further series of experiments conducted on a large scale under green-house conditions.

It is, however, evident that nicotine and lead arsenate sprays are both effective if applied immediately after the larvae have emerged and before they have begun to spin up. The chief difficulty experienced was that of reaching the young larvae with the nicotine spray, while in the same way it was not easy to coat the entire plant with lead arsenate. Unavoidably many leaf surfaces (those spun together) were not covered with the poison. The older the larvae the more serious was this difficulty. Both upper and lower surfaces of the leaves require to be sprayed. It is apparent that systematic and continued spraying with a nicotine spray from the time the first eggs of the year are expected to hatch (end of March) would account for large numbers of young larvae. Owing to the marked overlapping of broods in this species, the spraying must be carried on at intervals (say once per week, plants thoroughly sprayed) throughout spring, summer and autumn.

While the results of the arsenical spray are less rapid and less apparent than those of the contact insecticide, yet, since the larvae wander during feeding it is obvious that if the plants be periodically sprayed the death of many will eventually result as has been shown in the above experiments.

Gibson and Ross (1922) found a contact spray successful for the control of *Phlyctaenia ferrugalis* and so it would also appear that a nicotine spray is to be recommended in the case of *T. pronubana*. In the experiments at Kew it was found that spraying with any insecticide is useless unless the young larvae (1st and 2nd stages) are tackled: the spinning habit of the species makes it impossible to reach the larvae or the leaf surfaces on which they are feeding at later stages.

Fumigation with hydrocyanic acid gas is of no use in dealing with the pest. Molinas (1914) found that fumigation with nicotine gave no results and after experimenting with sulphuric acid and hydrocyanic acid gas finally abandoned any type of fumigation as successful control measures.

At Kew the larvae have been destroyed and their numbers successfully reduced by hand-picking. While this is a comparatively easy matter when the larvae are half to full grown, the younger stages are not easily seen and are thus usually passed over. Systematic destruction of larvae, pupae and egg-masses—the latter being frequently found on the "lights" of green-houses—are all simple, practical means of reducing the numbers of the pest under glass. It is, however, futile to expect to eradicate entirely *T. pronubana* from green-houses in a locality such as Kew, where the species is found abundantly in the open in their immediate vicinity. Mention has already been made (p. 431) of the suggested use of muslin

or wire gauze screens to fit all openings in the green-houses and so prevent entrance and exit of adults. If this be done and systematic spraying and examination of plants be carried out before transferring from house to house, the pest would, as far as can be seen at present, be eradicated from infected green-houses.

In conclusion, it may be remarked that at Kew, *T. pronubana* has not proved so troublesome during 1923 as during 1921 and 1922 when this work was first begun. Hand-picking has been carried out with success throughout the period.

V. PARASITES OF *T. PRONUBANA*.

Millière (1859) describing the life-history of *T. pronubana* on the Continent, states that his observations showed the species to be parasitised to an extent of 75 per cent. by a Tachinid *Morinia bigoti*, Robineau.

Adkin (1907), working in this country, in his first observations did not obtain a single parasite. During summer, 1908, however, he reared several specimens of a Tachinid—*Zenillia roseana*, B. and B.—from larvae and pupae of *T. pronubana*. The writer has recently been in communication with Adkin on this question of parasites and with his permission quotes the following extract from a letter he has received and in which Adkin records the Ichneumon *Oedematopsis scabricula*, Grav. as a parasite of *T. pronubana*:

"...in 1907 I turned a lot of *T. pronubana* eggs out on an euonymus bush in my garden at Lewisham. In the spring of 1908 I searched the bush for larvae but could find only two and each of them had a parasitic larva attached to it. I cannot say whether there had been more of the Tortrix larvae and that they had been killed by the parasites or not, but these two were eventually sucked dry by them. I sent the parasites which I reared from these two to Claude Morley and he identified them as the Ichneumon, *Oedematopsis scabricula*, Grav. The parasites attach themselves to their hosts externally and the best description I can give of their appearance is that they are curled between the 2nd and 3rd segments or perhaps 3rd and 4th. Larva found with parasite attached—April 12th.

Parasite emerged—May 29th and June 9th."

An Hymenopterous larva was found feeding on a larva of *pronubana* just as Adkin describes above on April 13th, 1923 on a privet hedge at Richmond. Unfortunately the parasite did not attain maturity but it is probable that this was the same species of Ichneumon as Adkin obtained.

In the observations made under glass at Kew several specimens of

two (probably) Hymenopterous parasites of *pronubana* have been obtained but never have any Tachinid parasites been found. In August and September, 1921, three Ichneumons issued from pupae of *pronubana*—the only parasites obtained during the summer of that year. In 1922 no parasites were found, although a thorough search was made in the houses at Kew and 30 or 40 pupae were collected from all of which moths emerged. In spring, 1923, eight Hymenopterous cocoons were collected from plants in the green-houses; beside each cocoon a shrivelled larval skin was found. During April and May the adult parasites emerged and an experiment was commenced to follow out their life-history. Owing to lack of material, however, this had, for the present, to be abandoned. The parasites obtained have been submitted to Dr Waterston of the British Museum and he has promised to identify them. At present, however, the names of the species have not been received. It will be interesting to see whether they are the same as those reared in the open by Adkin. Owing to the extremely small number of parasites obtained at Kew it is quite impossible to give any figures representing the percentage of parasitism.

It would appear that, up to the present, *T. pronubana* is singularly free from parasites in Britain. Since the species is a native of foreign shores and is not indigenous to this country it has evidently been introduced without the parasites which attack it so successfully and, according to Millière, in such numbers in its native habitat. Hence as a natural means of control, parasitism must be regarded at present as of little importance, especially since parasitised larvae, and therefore parasites, as well as unparasitised larvae are destroyed by hand-picking.

VI. *TORTRIX PRONUBANA* IN RELATION TO DYAR'S HYPOTHESIS.

Dyar (1890) in a paper on the "Number of Moults of Lepidopterous Larvae" establishes an hypothesis which he has found to hold good for all the species of Lepidopterous larvae he has examined. His hypothesis states that "the widths of the head of a larva in its successive stages follow a regular geometrical progression and if, in examining the measurements of heads taken in following out a life-history, any deviation from the calculated progression is shown, it is evidence that an error has been committed or that the larva has behaved in an abnormal manner." Dyar selected the head of the larva for measurement "as the part not subject to growth during the stage, and its width as the most convenient measurement to take."

He gives in this paper a list of species which shows the actual and

calculated larval head measurements together with the ratio existing between the measurements of successive larval heads. The following are a few examples to explain further Dyar's hypothesis. All measurements are in millimetres and are correct to within ·1 mm.

Species with Five Larval Stages.

Papilio cresphontes, Cram.

Calculated	·7, 1·1, 1·6, 2·3, 3·4
Ratio	·66
Found	·6, 1·1, 1·6, 2·2, 3·4

Datana palmii, Bent.

Calculated	·5, ·9, 1·5, 2·7, 4·6
Ratio	·58
Found	·5, ·9, 1·6, 2·7, 4·6

Species with Seven Larval Stages.

Halisidota harrisii, Walsh.

Calculated	·4, ·6, ·8, 1·2, 1·7, 2·4, 3·5
Ratio	·7
Found	·4, ·6, ·9, 1·4, 1·6, 2·3, 3·5 ·4, ·6, — 1·3, 1·7, 2·6, 3·6

It is probable that the figures Dyar gives are averages selected from a long series of measurements to illustrate the remarkable similarity between the measurements calculated and those actually found. In the figures given in Table XI for *T. pronubana* all measurements are given and it is thus apparent that whereas the general law holds, nevertheless there are considerable variations between measurements of similar stages.

The principle is of considerable importance when viewed from the point of establishing the number of moults for any given species and hints at the possibility of ascertaining the causes of variation in a life-history.

During summer, 1922, whilst conducting experimental work on the biology of *T. pronubana*, it was decided to investigate the larval stages and number of moults on the lines of Dyar's hypothesis. So far as has been observed it was found that growth in the species agreed with the results which Dyar found for other Lepidopterous larvae, namely, that the widths of the head of a larva in its successive stages follow a regular geometrical progression.

In the experiments at Kew the procedure was as follows: batches of larvae were reared on carnation plants and as the different moults

occurred the cast heads found after each moult were measured by means of an eye-piece micrometer. At the same time the breadth of the head of the newly-moulted larva was also obtained and in this way a series of measurements of larval heads and cast head capsules got for each instar. It was found impossible to follow an individual larva throughout its life because of laboratory conditions where numbers of larvae were kept together on a limited number of host plants and also since larvae, when disturbed, moved to another part of the plant so that it was not always possible to obtain with certainty the same individual that had been measured in preceding instars.

Measurements of larval heads were taken at the broadest part; it was found that if cast heads were mounted in balsam on glass slides before measurement the weight of the cover glass caused the head to split and give too large a reading. This was especially the case in the later instars. Accordingly it was found most satisfactory to measure all cast head capsules without mounting, merely placing them dry on a microscopic slide and measuring in the usual manner with the use of an eye-piece micrometer.

The breadth of larval heads of similar stages was found to vary slightly as can be seen in Table XI given below. The variation, however, was not sufficient to upset Dyar's hypothesis. The ratio between the breadth of larval heads of *T. pronubana* in any two successive instars was 1·37. For example, the average breadth of a 1st stage larval head was ·24 mm. and that of a 2nd stage larval head ·33 mm. The ratio ·33 : ·24 is thus 1·37. Having obtained this ratio it is possible to calculate the size of the larval head of any other instar. Further, it enables one to determine the exact stage of growth of any given larva found at random in the green-houses or elsewhere.

During 1922 the summer brood larvae underwent seven moults, the pupa appearing after the seventh moult. In October an interesting observation was made: the cast head of a recently-moulted larva of the summer brood was compared with that of the larva itself and it was found that, while the cast head (·68 mm. broad) indicated 4th stage, the head of the larva itself indicated 6th stage (1·1 mm. broad). It is thus apparent that the 5th stage of this caterpillar had been entirely omitted and at moult IV instead of getting a 5th stage larva a larva corresponding to a true 6th stage was obtained. It must be borne in mind that the duration of the first four instars of the life of this larva had extended over an unusually long period. The result of this appears to have been elimination of the usual fifth moult and the production of a 6th stage

larva at moult IV. Further similar cases of delayed development resulting in the omission of a moult (omission of more than one moult was never recorded) were observed during autumn, 1922.

The conclusion may thus be drawn that, whereas a larva may prolong its life to a considerable extent, in so doing it undergoes fewer moults than a larva undergoing more rapid normal development. This would seem to be the direct result of the slow growth itself which would appear to render frequent moults unnecessary. It forms evidence to show, as has been frequently stated, that changes of skin or moults are the result of growth.

Table XI.

Head Measurements of Larvae of T. pronubana, *Hb. in Successive Instars.*

Stage	Micrometer measurements of larval heads (mm.)	Micrometer measurements of cast head capsules (mm.)	Calculated measurements (mm.)
1st*	·22, ·23, **·24**, **·24**, **·24**, ·26	·31, ·31, ·31, ·32	·24 = ·24
2nd*	·30, ·31, **·33**, **·33**, **·33**	·41, ·42, ·42, ·43, ·44, ·44	·24 × 1·37 = ·33
3rd	**·44**, **·46**, **·46**, **·46**, ·48, ·51, ·55	·46, ·58, ·66*, ·65*, ·67*	·33 × 1·37 = ·45
4th	**·62**, **·62**, **·62**, ·63, ·63, ·65, ·67	·60, ·60, ·62, ·62, ·65, ·68, ·73	·45 × 1·37 = ·61
5th	·78 (?), **·82**, **·84**, ·87, ·88	·80, ·81, ·84	·61 × 1·37 = ·83
6th	·94, ·97, 1·0, **1·1**, **1·1**, **1·1**, 1·2	·90, ·97, 1·04, 1·1, 1·1	·83 × 1·37 = 1·13
7th	1·2, 1·3, 1·4, **1·5**, **1·5**, **1·5**	1·5, 1·2	1·13 × 1·37 = 1·54

* Denotes cast head capsules mounted in balsam before measurement—hence large readings.

Ratio, calculated, = 1·37.

SUMMARY.

The moth, *Tortrix pronubana*, Hb., has lately proved troublesome in the glass-houses at the Royal Botanic Gardens, Kew. Its life-history has been studied and shows two main broods in the year. The periods of duration of the various larval stages and of the pupal stage have been studied in detail.

A description of the external morphology and chaetotaxy of the larva is given. Setal maps are given showing the distribution of the setae on the thoracic and abdominal segments of the 1st stage and full-grown larvae. The chaetotaxy of the larval stages is similar except in the 1st stage. The growth of the larva throughout its various stages, seven in number (normally six moults), conforms with Dyar's hypothesis that "the widths of the head of a larva in its successive stages follow a regular geometrical progression and if, in examining the measurements of heads taken in following out a life-history, any deviation from the calculated rogression is shown, it is evidence that an error has been committed or that the larva has behaved in an abnormal manner."

The chaetotaxy of the abdominal segments of the pupa is described and sex characters are noted.

A series of experiments on the control of *T. pronubana* under glass shows that spraying with nicotine or lead arsenate is effective if applied before and immediately after the eggs hatch. It is essential that the spray should be applied before the larvae commence to spin their webs. Destruction of the larvae by hand-picking is effective but the minute 1st and 2nd stage larvae are easily overlooked. Hand-picking combined with regular spraying is to be recommended as the best method of control.

Fumigation with hydrocyanic acid gas was found ineffective against larvae.

The screening of windows and ventilators with gauze or muslin is recommended as a check on the infestation of clean houses.

Two Hymenopterous parasites, not yet identified, were bred from larvae and pupae of *T. pronubana* but the percentage of parasitism was extremely low.

A list of host plants of *T. pronubana* under glass and in the open is given in an appendix.

In conclusion the writer wishes to express his indebtedness to Dr J. W. Munro for his advice and encouragement during this work which was carried out under his supervision. Thanks are due to the Director of the Royal Botanic Gardens, Kew, for the many facilities which were so willingly granted for experimental work and for general observations in the green-houses.

APPENDIX.

HOST PLANTS OF *T. PRONUBANA*, HB. UNDER GLASS AT KEW.

* *Denotes plants on which larvae are most abundant.*

Acacia, L. Leguminosae
A. armata, R. Br.
A. baileyana, F. Muell.
A. cultriformis, A. Cunn.
A. Drummondii, Benth.
*A. longifolia, Willd.
Var. mucronata
A. neriifolia, A. Cunn.
A. platyptera, Lindl.
A. pravissima, F. Muell.
A. pulchella, R. Br.
A. riceana, Hernel.

Agapetes, G. Don	Vacciniaceae
A. Moorei	
Albizzia, Durazz	Leguminosae
A. Julibrissin, Durazz	
Asparagus, Tourn.	Liliaceae
A. sprengeri, Regel.	
Boronia, Sm.	Rutaceae
B. elatior, Barth.	
Bucklandia, R. Br.	Hamamelideae
B. populnea, R. Br.	
Callistemon, R. Br.	Myrtaceae
C. Cunninghamii, Hort.	
C. salignus, DC.	
Calythrix, Labill.	Myrtaceae
C. tetragona, Labill.	
Cestrum, L.	Solanaceae
C. aurantiacum, Lindl.	
(Larvae yellowish in colour on this plant.)	
Chorizema, Labill.	Leguminosae
*C. ilicifolium, Labill.	
Clianthus, Banks & Sol.	Leguminosae
Species not determined.	
Coleonema, Barth. & Wendl.	Rutaceae
C. albuma, B. & W.	
*Coronilla, L.	Leguminosae
C. glauca, L.	
Var. variegata	
Cytisus, L.	Leguminosae
*C. fragrans, Lam.	
C. filipes, B. & W.	
Daphne, L.	Thymelaeaceae
D. odora, Thumb.	
*Dianthus, L.	Caryophyllaceae
Various species	
Erica, L.	Ericaceae
E. bowieana	
Eriostemon, Sm.	Rutaceae
E. buxifolius, Sm.	
E. scaber, Paxt.	
Geranium, L.	Geraniaceae
Species not determined	
Grevillea, R. Br.	Proteaceae
G. alpina, Lindl.	
G. oleoides, Sieber.	
G. ornithopoda, Meissn.	
G. punicea, R. Br.	
G. robusta, A. Cunn.	
G. thelemanniana, Hugel.	
Helichrysum, L.	Compositae
H. rupestre, DC.	
Lhotskya, Schan	Myrtaceae
L. ericoides, Schan.	

Mitraria, Cav. Gesneraceae
M. coccinea, Cav.
Nandina, Thmb. Berberideae
*N. domestica, Thb.
(Larvae markedly yellow on this plant)
Pimelea, Banks & Sol. Thymelacaceae
P. ferruginea, Labill.
P. Gnidia, Willd.
Var. pulchella
P. ligustrina, Labill.
Poinciana, L. Leguminosae
P. Gilliesii, Hook
Psoralea, L. Leguminosae
P. pinnata, L.
Rhododendron, L. Ericaceae
R. Edgeworthii, Hook
R. formosum, Wall.
Var. Gibsonii
R. Oldhamii, Maxim
R. Schlippenbachii, Maxim.
Tricuspidaria, R. & P. Tiliaceae
T. lanceolata, Miq.

The writer is indebted to Mr J. H. Durrant for the following list of plants from which *T. pronubana*, Hb. has been recorded in the open in Britain and abroad:

Arbutus, L. Ericaceae
A. unedo, L.
Aristolochia, Tourn. Aristolochiaceae
Species not determined
Asphodelus, L. Liliaceae
A. ramosus, L.
Cneorum, L. Simarubaceae
C. tricoccon, L.
Cytisus, L. Leguminosae
C. linefolius, Lam.
Daphne, L. Thymelaeaceae
D. gnidium, L.
Digitalis, L. Scrophulariaceae
D. obscura, L.
Euonymus, L. Celastrineae
E. japonicus, L.
Euphorbia, L. Euphorbiaceae
Species not determined
Geranium, L. Geraniaceae
Species not determined
Jasminum, L. Oleaceae
Species not determined
Ligustrum, L. Oleaceae
Species not determined
Lotus, L. Leguminosae
L. rectus, L. and other species

Mercurialis, L.	Euphorbiaceae
M. elliptica, Lam.	
Olea, L.	Oleaceae
O. europaea, L.	
Passerina, L.	Thymelaeaceae
Species uncertain	
Pistacia, L.	Anacardiaceae
P. Lentiscus, L.	
Rhus, L.	Anacardiaceae
R. Coriaria, L.	
Robinia, L.	Leguminosae
R. pseudacacia, L.	
Rosmarinus, L.	Labiatae
R. officinalis, L.	
Rubus, L.	Rosaceae
Species not determined	
Scabiosa, L.	Dipsaceae
Species not determined	
Smilax, L.	Liliaceae
S. aspera, L.	
Solidago, L.	Compositae
Species not determined	
Thymus, Tourn.	Labiatae
T. vulgaris, L.	
Viburnum, L.	Caprifoliaceae
V. tinus, L.	

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EXPLANATION OF PLATE XV.

Carnation plants, showing injury resulting from attack by *T. pronubana*.

Plant A. Healthy unattacked plant.

B. 4–5 weeks after hatching of eggs on plant.

C. 6–8 weeks after emergence of young larvae; plant dying.

(*Received March 18th*, 1924.)

CERTAIN ASPECTS OF THE DAMAGE TO OATS BY THE FRIT FLY

By J. C. F. FRYER and J. E. COLLIN.

(With 3 Text-figures.)

Foreword. Since 1918 research into various problems connected with Frit Fly (*Oscinella frit* L.) has been in progress at the Ministry's Pathological Laboratory, the investigations having in some instances been made in collaboration with workers at other institutions. As the chief function of the Ministry's laboratory is not research, the work has been carried on in an intermittent manner and has resulted in the accumulation of much unpublished material which—if time had permitted—would have formed the basis for several progress reports instead of having been used merely as a stepping-stone from one experiment to another. Many of these earlier experiments have now served their purpose and are already out of date, but the following study, which deals in the main with the attack of the second brood of the Frit Fly on the oat grain, may still prove of interest and also serve as an introduction to other work carried out more recently.

The opportunity may be taken of expressing a debt of gratitude to all those who have assisted us directly or indirectly—notably to Mr Roebuck of the Midland Agricultural College, to Mr Robson of the East Anglian Agricultural Institute, to Mr Cecil King of Long Sutton, and the Lord Wandsworth Trustees who kindly gave facilities for the experiments to be carried out on their estate.

A. *The Damage to the Oat Kernel.* In 1918 plots were sown at Long Sutton, Hants., with "White Abundance" and "Black Tartarian" oats on March 18th and April 25th, one plot of each variety and of each sowing being left as control, a second plot receiving 1 cwt. per acre of sulphate of ammonia at the time of sowing, and a third being top-dressed with nitrate of soda on June 9th when the Frit attack on the young plant was causing obvious signs of distress in the crop. So far as this attack on the young plant is concerned, it may at once be stated that the value of early sowing was conclusively shown, but no differences

justifying any deductions arose out of the manurial treatments or their absence. At harvest, large samples were gathered from different points scattered over each plot and the resulting grain was then spread out on a flat surface to a depth of one kernel only. The grain-covered area obtained in this way was again sampled, every grain being taken from equal-sized "plots" in different parts of the whole area. This second sample, consisting of 400–1000 grains, was examined by opening each grain in order to discover which were sound, which had been damaged by Frit and which were "blind," the results being as follows:

White (*Abundance*)

Sowing	Plot	Grains examined	% sound	% frit	% blind
Early	Control	430	90	5·8	4·2
	S. of A.	641	90·5	5·6	3·9
	N. of S.	1211	89·7	5·1	5·2
	Average all plots	2282	90	5·4	4·6
Late	Control	497	74·3	16·1	9·6
	S. of A.	937	73·3	19·3	7·4
	N. of S.	1029	70·7	17·2	12·1
	Average all plots	2463	72·4	17·8	9·8

Black (*Tartarian*)

Sowing	Plot	Grains examined	% sound	% frit	% blind
Early	Control	1342	92·1	3·2	4·7
	S. of A.	1095	89·1	4·1	6·8
	N. of S.	780	89·5	5·7	4·8
	Average all plots	3217	90·5	4·1	5·4
Late	Control	1070	76·5	14·9	8·6
	S. of A.	957	73·5	14·8	11·7
	N. of S.	852	66	21	13
	Average all plots	2879	72·4	16·6	11

The loss in grain which is undoubtedly due to Frit thus works out at 4 per cent. at least in the early-sown and 16 per cent. in the late-sown, while there were in addition a certain number of "blind" or infertile spikelets, that is to say, spikelets in which the grain had never developed, though outwardly they might appear practically normal. The cause of this blindness is unknown; it may in some cases be due to Frit but it can seldom be attributed to this pest with any certainty and blind grains are therefore counted separately in all the subsequent tables. Evidently late sowing renders the crop more susceptible to the second brood of Frit while it appears to influence, though to a less extent, the percentage of blind spikelets.

The considerable loss in sound grain indicated by this experiment, suggested that the loss to the country due to the second brood attack of the fly had not been sufficiently realised and arrangements were made to obtain samples at harvest from oat crops throughout England and Wales. As a result, in 1918 183 samples were received from 30 different counties, and in 1919 161 samples from 39 counties. The grain was dealt

Oat Trials, 1918 *and* 1919. *Sampling of Grain for Frit Attack.*

White Oats

County	1918 (%)				1919 (%)			
	Cases	Sound	Frit	Blind	Cases	Sound	Frit	Blind
Northumberland	—	—	—	—	6	93·1	0·4	6·5
Durham... ...	2	87·5	3·3	9·0	—	—	—	—
Cumberland ...	—	—	—	—	4	95·7	0·6	3·7
Yorks.	4	89·3	3·0	7·7	6	84·9	7·8	7·3
Lancs.	11	86·0	7·5	6·5	7	83·85	9·45	6·7
Cheshire ...	4	87·8	5·7	6·5	4	83·4	10·9	5·7
Lincs.	3	78·5	15·6	5·9	—	—	—	—
Notts.	1	92·7	4·6	2·7	4	45·2	38·5	16·3
Derby	8	89·85	5·3	4·85	1	83·0	13·5	3·5
Leics.	—	—	—	—	1	62·5	9·5	28·0
Staffs.	1	91·6	2·7	5·7	3	73·9	22·8	3·3
Salop	11	65·1	24·8	10·1	4	69·4	20·0	10·6
Warwicks. ...	6	83·8	11·1	5·1	1	43·2	30·0	26·8
Norfolk	3	83·2	12·6	4·2	1	84·6	7·5	7·9
Cambs.	5	87·1	9·15	3·75	1	65·4	24·3	10·3
Northants. ...	1	73·5	21·6	4·9	10	66·3	22·8	10·9
Suffolk	2	92·1	3·95	3·95	1	76·7	9·4	13·9
Essex	—	—	—	—	2	86·05	9·7	4·25
Beds.	6	77·2	18·0	4·8	3	71·9	16·3	11·8
Kent	1	93·5	3·9	2·6	3	69·76	23·26	6·96
Bucks.	—	—	—	—	1	66·3	29·8	3·9
Oxon.	3	86·8	9·0	4·2	3	68·7	16·7	14·6
Middlesex ...	—	—	—	—	1	79·1	15·2	5·7
Berks.	1	91·8	5·5	2·7	3	78·4	15·3	6·3
Hants. & I. of W.	9	85·4	8·5	6·1	3	64·4	18·6	17·0
Dorset	3	93·8	2·4	3·8	—	—	—	—
Worcs.	7	80·4	11·7	7·9	19	46·75	44·95	8·3
Hereford ...	—	—	—	—	1	65·7	27·3	7·0
Gloucester ...	5	87·0	10·25	2·75	—	—	—	—
Wilts.	3	91·3	3·8	4·9	—	—	—	—
Somerset ...	1	95·0	2·8	2·2	2	80·55	12·95	6·5
Devon	5	85·95	7·9	6·15	6	83·6	12·6	3·8
Cornwall ...	5	80·7	13·5	5·8	2	83·5	10·15	6·35
Anglesey ...	1	96·7	1·1	2·2	2	87·6	0·6	11·8
Denbigh ...	5	83·6	7·6	8·8	2	85·25	11·55	3·2
Flint	2	85·85	3·9	10·25	2	75·35	20·6	4·05
Carnarvon ...	3	91·63	2·93	5·43	7	84·8	6·3	8·9
Merioneth ...	3	89·86	1·76	8·36	—	—	—	—
Radnor	—	—	—	—	2	63·45	30·0	6·55
Cardigan ...	—	—	—	—	4	86·1	0	13·9
Brecon	1	81·5	10·6	7·9	—	—	—	—
Carmarthen ...	—	—	—	—	2	93·05	2·7	4·25
Glamorgan ...	1	83·3	7·5	9·2	—	—	—	—
Total for England and Wales	127	84·1	9·7	6·2	124	72·9	18·5	8·6

Oat Trials, 1918 *and* 1919. *Sampling of Grain for Frit Attack.*

Black Oats

County	1918 (%)				1919 (%)			
	Cases	Sound	Frit	Blind	Cases	Sound	Frit	Blind
Northumberland	—	—	—	—	2	96·45	—	3·55
Durham... ...	2	94·45	0·45	5·1	—	—	—	—
Yorks.	1	76·6	16·9	6·5	—	—	—	—
Lancs.	2	81·05	11·65	7·3	—	—	—	—
Cheshire ...	1	61·7	12·3	26·0	1	90·8	1·6	7·6
Lincs.	2	81·0	9·75	9·25	—	—	—	—
Derby	2	92·4	4·45	3·15	—	—	—	—
Warwicks. ...	2	55·65	36·8	7·55	2	63·6	30·2	6·2
Cambs.	2	82·95	11·55	5·5	2	73·65	12·0	14·35
Northants. ...	3	74·1	17·8	8·1	—	—	—	—
Suffolk	—	—	—	—	2	78·5	13·05	8·45
Herts.	—	—	—	—	1	91·0	2·8	6·2
Beds.	2	85·8	9·55	4·65	—	—	—	—
Kent	2	81·2	11·85	6·95	1	65·2	28·0	6·8
Surrey	1	92·3	4·2	3·5	—	—	—	—
Sussex	4	91·2	4·4	4·4	—	—	—	
Bucks.	1	85·6	8·2	6·2	—	—	—	—
Oxon.	2	91·8	5·0	3·2	3	62·4	28·0	9·6
Berks.	2	81·05	11·05	7·9	1	76·8	7·0	16·2
Hants. & I. of W.	10	90·0	4·1	5·9	—	—	—	—
Dorset	8	89·4	3·1	7·5	—	—	—	—
Worcs.	2	79·3	12·35	8·35	4	49·9	40·3	9·8
Gloucester ...	2	82·1	10·2	7·7	1	81·3	15·4	3·3
Wilts.	5	82·9	8·8	8·3	—	—	—	—
Somerset ...	3	82·6	8·0	9·4	3	70·0	16·4	13·6
Devon	7	81·2	8·1	10·7	6	77·6	16·8	5·6
Cornwall ...	12	86·55	5·55	7·9	2	89·1	0·25	10·65
Anglesey ...	2	91·1	0·5	8·4	1	92·4	3·7	3·9
Carnarvon ...	2	91·7	1·7	6·6	1	79·2	4·7	16·1
Radnor	—	—	—	—	2	90·6	4·85	4·55
Brecon	2	85·95	7·35	6·7	—	—	—	—
Monmouth ...	2	94·75	2·55	2·7	—	—	—	—
Glamorgan ...	1	93·9	0·2	5·9	2	71·25	24·75	4·0
Total for England and Wales ...	89	85·2	7·5	7·3	37	75·2	16·6	8·2

with in the manner indicated previously and the details so obtained are given in tables dealing respectively with White and Black oats (precise varieties mostly unknown).

It will be seen that the number of samples received from any one county or district was hardly sufficient to justify any numerical generalisation on a basis either of counties or districts, but it is evident that the material loss caused by the second brood attack is very great. Even in 1918, a season in which Frit Fly was less prevalent than usual, the actual loss in grain is heavy. One hundred and twenty-seven samples of white oats gave an average of 9·7 per cent. grain per sample damaged by Frit. In 1919—the following year—the attack was patchy, distinctly bad in certain counties but relatively light in others: on the whole, perhaps

slightly worse than the average. In that year 124 samples of white oats prove to have no less than 18·5 per cent. of grains per sample damaged by Frit, while in case it may be considered that this result is due to the receipt of an excessive number of samples from the areas most attacked, it may be mentioned that on these figures the county average works out at 15·8 per cent. per county. In each year, black oats appear slightly but definitely better than white oats, as in the case of the Long Sutton



Fig. 1.

experiment, but in view of the wide range shown by the figures (black oats in 1918 ranged from 0–36·8 per cent. and in 1919 from 0–40 per cent.) it would not be safe to assert on these figures alone that the difference is significant.

The information gained in 1918 caused greater attention to be paid in 1919 to the grain attack, and in addition to obtaining the statistics given in the previous tables, arrangements were made to carry out

experiments at three centres to attempt to discover something further as to the factors influencing the attack.

Previous experiments seemed to indicate that there was a critical period in the blossoming or early stage of the oat-panicle when the plant was most susceptible, and also a period of greatest prevalence of the brood of Frit Flies responsible for the attack, with the result that maximum infestation occurred when the two coincided. It was therefore decided to screen plants at intervals during the development of the seed-head and to release periodically others that had been screened prior to the emergence of the panicle and so attempt to prove or disprove the above supposition. The screening was done by means of tiffany bags supported by stakes (Fig. 1), and each set of plants screened or released was labelled with the date when screened or exposed, the panicles being carefully collected at harvest and every grain examined for Frit infestation. This method needed excessive care in the fixing of the bags, and proved troublesome, the attention of mice, rats, and dogs being attracted to the bags and of children to the useful sticks by which they were supported. In consequence the results obtained at two centres are too irregular to quote in detail, but the Long Sutton experiments were more fortunate, although even here a young retriever rendered certain of the baggings abortive, while in one or two cases rats or mice nibbled holes in the tiffany and may have allowed Frit Flies to enter. Fortunately few of the accidents occurred in connection with the control plants, that is to say, those covered from a date prior to the emergence of the ear from the sheath until harvest, and Frit Fly injury was absent from these ears covered throughout the experiment except in the case of one bag on Plot II which had been disturbed by the dog. It is therefore clear that the bags were applied at a sufficiently early date. Further, the oats within the bags developed normally without differing appreciably from the normal uncovered plants.

The details of the experiment were as follows:

Plots, not measured but exceeding 1 acre, were sown:

Plot I. March 28th,
Plot II. April 17th,
Plot III. ,, 24th,
Plot IV. May 15th,

the irregularity in date being due to weather conditions and cultural difficulties. Later work in connection with the "bagging" was carried out as follows:

June 18*th*. Plot I. Attached 15 bags.
Plot II. „ 3 „
Plot III. „ 15 „
Plot IV. „ 3 „

On this date the top spikelet of the panicle was just visible within the leaf sheath on one or two stems on Plot I, but such stems were not bagged. No panicles were visible on the other plots and the plants on Plot IV were only about 6 inches high and difficult to enclose. On this date also the plots were sampled as follows in order to obtain some measure of the first brood attack. Ten stations, approximately equidistant, were selected in each plot and at each station one foot of row was chosen at random (so as to avoid any unconscious selection of good or bad plants) and the oats from the measured length dug up and removed to the laboratory for examination. The average number of plants per foot run of row was approximately 15 (extremes = 14·7 and 16·9) and the results as follows:

	Percentage of sound plants	Percentage of plants attacked but sending up stems bearing panicles	Percentage seriously attacked, not sending up stems bearing panicles
Plot I	77·7	16·9	5·4
„ II	89·0	4·9	6·1
„ III	87·6	0·6	11·8
„ IV	54·4	12·9	32·7

It is difficult to suggest what reliance can be placed upon these figures as the experimental error may be very large; on the other hand, any serious attempt to ensure accuracy would have required a large staff of investigators, while the consequent interference with the crop might not improbably have prevented the main experiment. Accepting the figures as they stand, column 3 coincides with expectation and with the appearance of the crops in the plots. Column 2 lends some support to the theory that it is possible to sow the oat crop too early, for Plot II, which gives the best results (in at least so far as the number of young plants not attacked by flies of the first brood is concerned), also, as will be seen subsequently, came out well in the grain attack. It is not proposed, however, on account of the difficulty in estimating first brood attack, to base detailed deductions on these figures and they are inserted mainly in order to preserve the records complete.

July 1*st*. Plot I. Attached 4 bags. Released 4.
Plot II „ 3 „
Plot III. „ 4 „ „ 4.
Plot IV. „ 3 „

On this date the panicles were clear of the sheath on Plots I, II and III, and were breaking out on Plot IV. The following notes were made as to the conditions in detail:

Plot I. [1]Upper spikelet, 1st and 2nd grains fertilised.
Middle spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Lower spikelet, 1st grain not fertilised.

Plot II. Upper spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Middle spikelet, 1st grain pollen just shed, 2nd grain not fertilised.
Lower spikelet, 1st grain quite immature.

Plot III. Upper spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Middle spikelet, 1st grain not fertilised.
Lower spikelet, 1st grain quite immature.

Plot IV. Upper spikelet, 1st grain pollen just ready to shed, 2nd grain immature, and remainder very immature.

On *July 11th and July 25th* the same procedure was followed both as regards numbers of bags put on and bags taken off in the case of each plot. On July 11th the following notes were made as to the conditions of the kernel:

Plot I.	Upper spikelet, Middle ,,	Grains milky and full size.
	Lower ,,	Grains half size.
Plot II.	Upper spikelet	Larger grains milky.
	Middle ,,	Grains hardly full size.
	Lower ,,	Grains quite small.
Plot III.	Upper spikelet	Larger grains becoming milky.
	Middle ,,	Grains small (under half size).
	Lower ,,	Grains quite small.
Plot IV.	Upper spikelet	Grains half size.
	Middle ,,	Grains quite small.
	Lower ,,	Grains not fertilised.

By July 25th the plots were turning colour, Plots I and II being nearly ripe, and no easy method of differentiating between the condition of the grain in the different plots was found. On August 6th the

[1] The terms "upper," "middle," and "lower" refer to the position of the spikelet in the panicle, the "upper" being the terminal spikelet, the "lower" a spikelet borne upon a lowermost branch of the panicle, and the "middle" a spikelet as near as possible halfway between upper and lower. The 1st grain refers to the larger of the two grains usually found between each pair of glumes, the 2nd grain being the smaller one arising from near the base of the first.

"bagged" and "released" plants were judged ripe and were removed to the laboratory for detailed examination, which gave the following results:

Date	Plot I			Plot II		
	No. of bags	Total no. of grains	% attacked by frit	No. of bags	Total no. of grains	% attacked by frit
Bagged Throughout (control)	3	820	0	2*	429	0·2
July 1	4	1208	1·9	3	784	1·5
„ 11	4	1625	2·7	3	934	2·5
„ 25	4	1897	2·2	3	1254	2·3
Released						
July 1	4	807	0·8	—	—	—
„ 11	4	1065	0	—	—	—
„ 25	4	888	0	—	—	—

Date	Plot III			Plot IV		
	No. of bags	Total no. of grains	% attacked by frit	No. of bags	Total no. of grains	% attacked by frit
Bagged Throughout (control)	3	466	0	3	382	0
July 1	3*	532	1·1	3	638	0·15
„ 11	4	894	2·9	3	798	3·3
„ 25	4	936	2·4	3	1115	9·7
Released						
July 1	3*	647	2·5	—	—	—
„ 11	4	528	0·3	—	—	—
„ 25	4	510	0	—	—	—

* One bag in each of these cases was destroyed by a dog and is not included.

In the case of the control in Plot II it may be mentioned that the dog completely destroyed one bag and disturbed another sufficiently to account for the 0·2 per cent. infestation. For convenience in discussion the above figures have been reduced to the form of a chart and with Mr Cunliffe's permission a curve showing the prevalence of Frit Flies at Oxford between July 7th and August 6th, 1919 has been added(1).

Certain points are shown very clearly by the graph (Fig. 2):

(I) *Two factors were operating throughout the experiment, the first being a variation in the state of susceptibility of the oat to an attack, and the second a variation in the number of flies present on the plots.*

This was to be expected but the definite demonstration was not without value and special interest attaches to the proof that the appearance of the second brood of the fly was well defined and was not swamped by the overlapping of the two broods. It will be noticed that the maximum

infestation on Plots I, II and III does not rise above 29 per 1000 and no increase of infestation took place after July 11th, while on Plot IV the figure of 97 per 1000 was recorded on July 25th. It is therefore practically certain that the oats on Plots I, II and III had passed the stage of greatest susceptibility before the great increase in the number of flies (due to the emergence of the second brood) began about July 11th. On the other hand, the fact that the curves showing the increase in infestation on Plot IV and the increase of flies at Oxford are more or

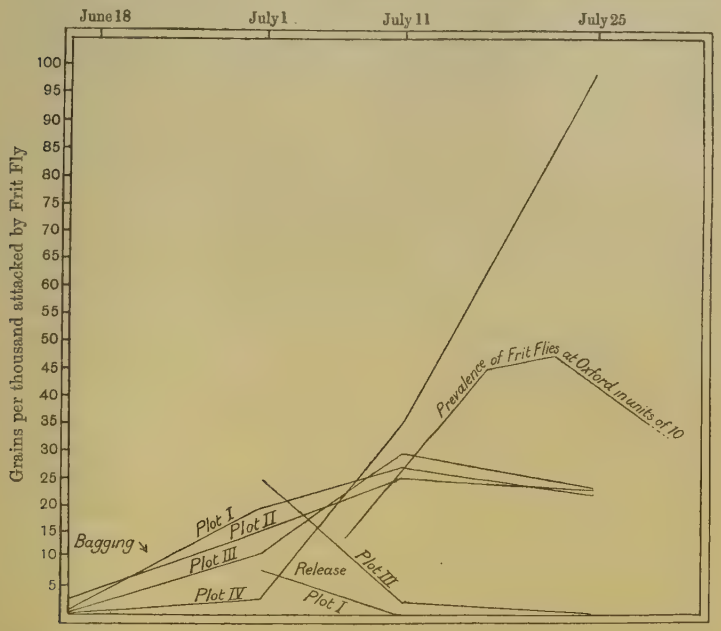


Fig. 2.

less parallel, equally suggests that in this case the period of maximum susceptibility of the plant coincided with the maximum prevalence of the flies.

The deduction as a whole is satisfactory as it indicates the possibility of "dodging" the grain infestation by appropriate timing of the crop exactly as is being attempted in the case of the first-brood attack on the young plant, earliness in each case being the great desideratum.

(II) *The period of maximum susceptibility of the oat spikelet lies approximately at the time of fertilisation.*

As mentioned above, by July 11th, Plots I, II and III had reached their maximum infestation and were practically no longer susceptible to infestation for the attack of 3 per 1000 obtained in the oats released on Plot III on this date may well be due to a small retarding influence on the plants owing to their having been bagged since June 18th: it is in any case too slight to invalidate the deduction. On the other hand, Plot IV on July 1st was not appreciably infested although the other plots prove that sufficient flies were available to cause an infestation up to 19 per 1000. It may be concluded therefore that the oats were most attractive to the flies for the purpose of oviposition between the stages represented by the oats on Plot IV on July 1st and the oats on Plot III on July 11th.

On July 1st the most forward grain in the panicles on Plot IV showed pollen just ready to be shed, while on July 11th the most backward grains on Plot III, though fertilised, were still quite small—at any rate under half their ultimate size. From this it might be deduced that the condition of maximum susceptibility (to oviposition) in the oat lies between fertilisation and the time when the oat kernel is half-size, a period which, in these experiments, cannot have lasted for much more than a week. It should, however, be noted that up to July 1st the oats on Plot I had been more heavily infested than those on Plot III, while immediately after this date (as proved by the released bags) the position was reversed. From this it would appear that the maximum susceptibility to attack of the oats on Plot I occurred just before July 1st and for those on Plot III just after. In view of the state of the oat panicles about this date, the above consideration would appear to limit still more closely the stage of maximum susceptibility to the time of fertilisation (for the spreading of the time of infestation over several days might be largely accounted for by the fact that oats in a panicle do not all arrive at a state for fertilisation at the same time). The period of susceptibility is thus surprisingly short and perhaps can hardly be accepted as a result of one year's experiments only. Its commencement is indicated by Plot IV only and is based on an examination of 638 grains, and it is possible that a larger experiment might indicate a degree of susceptibility prior to fertilisation. In this connection reference should be made to Roebuck's observations(2) on the occurrence of Frit larvae feeding in the oat panicle prior to its emergence from its sheath—which would appear to be entirely inconsistent with the results obtained in the present experiment. However, it would seem clear that an explanation of Roebuck's observations must be sought along lines

other than by advancing to an earlier stage the beginning of the true period of grain susceptibility. At Long Sutton, where oat plants were protected from Frit Fly throughout, 11 bags containing 1911 grains had only one grain attacked by *Frit* and even this one is explicable on the grounds of experimental error (the dog) rather than by assumption of infection prior to "bagging." That absence of flies was not responsible for this result is proved by the fact that Plots I, II and III became infested as soon as flowering occurred—Plot IV in a less advanced stage being hardly attacked. It is not therefore considered that Roebuck's results necessitate any great advancement of the stage of susceptibility of the oat grain prior to flowering of the oat. It may be asked whether Roebuck's results are not explicable by assuming that infestation took place at the close of the first attack on the young plant, which in his case may have been prolonged to a later date than usual, and to have included the stage when the plant was about to throw up the flowering stem. The larvae would then have been carried up with the "ear" with the result he has recorded. The parents of these aberrant larvae may, as he suggests, have been second brood flies emerging earlier than usual or late first brood flies, but so far as the oat is concerned, this appears to be immaterial.

Turning now to the end of the stage of susceptibility of the oat grain, the evidence available is considerable. Plots I, II and III on July 11th comprised grains varying from "half-sized" to "small" but none were in the recently fertilised condition. By the bagging experiments no increase in the infestation appears to have occurred after July 11th and this result is fully confirmed in the case of Plot I by the release experiments—panicles released on that date escaping attack altogether. The panicles of Plot III, also released on July 11th, comprising 528 grains proved attacked only to the extent of 3 per 1000—an attack which in any case is sufficiently low to be regarded as substantially in agreement with the bagging experiments. The slight difference is, however, explicable on the assumption that the bags which had been on since June 18th had slightly delayed the progress of ripening and so held a few grains in a susceptible condition for a day or two longer than the average for the plot. It is clear that if Plots I, II and III had been to any extent susceptible they must have been attacked, since Plot IV after July 11th showed an increase of 64 damaged grains per 1000 between this date and July 25th, proving the presence of ample flies. It would therefore seem clear that the oat grains on these experimental plots were at the latest no longer susceptible by the time they had

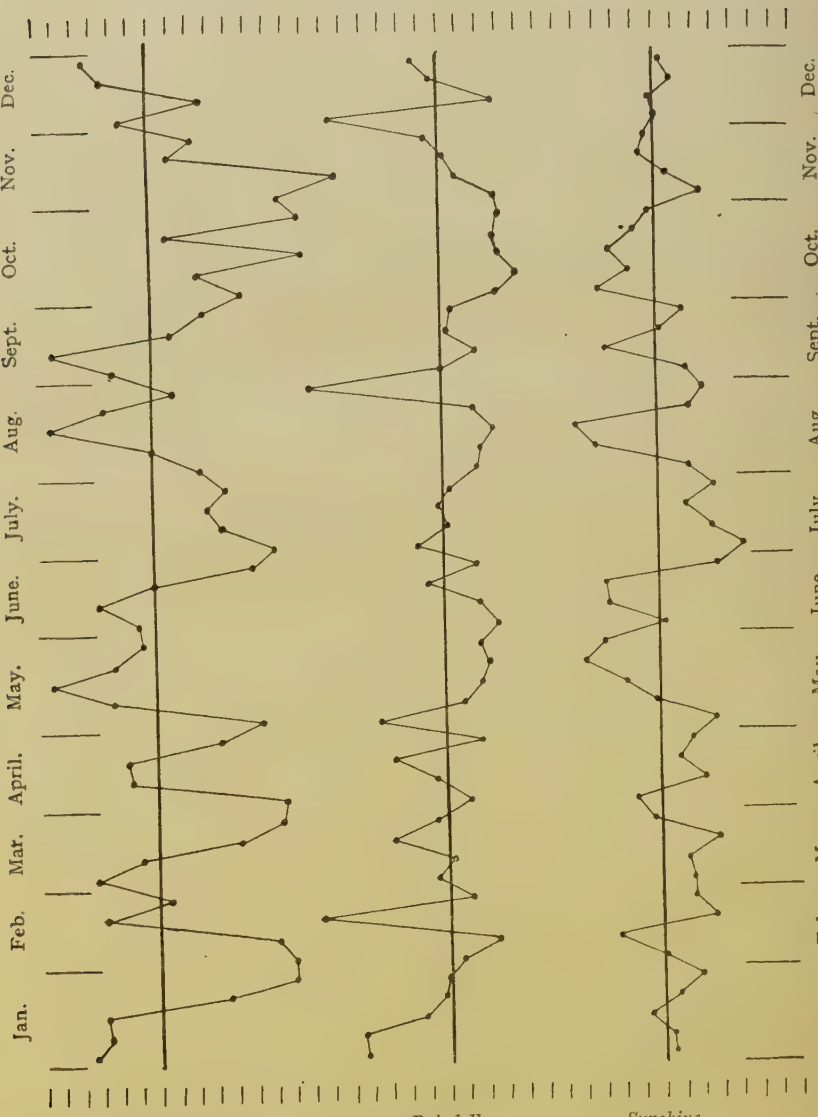

Jan.
Feb.
Mar.
April.
May.
June.
July.
Aug.
Sept.
Oct.
Nov.
Dec.
Temperature
Scale :—(Degrees Fahrenheit).
Rainfall
(5 Millimetres).
Sunshine
(Hours).

Fig. 3.

swelled to half their full size and there is at present no adequate reason for not extending this deduction to oats of the Abundance varieties in general, if not to all varieties. As the maturation of the grain is influenced by weather conditions, it may be enquired as to whether July, 1919 conduced to abnormally rapid ripening. This, however, can hardly have been the case as the month was cooler than the average, with less sunshine and about a normal fall of rain as is shown in the accompanying weather chart[1] for S.E. England, where the variation above and below the average mean is given for temperature, rainfall and sunshine. Finally, before leaving this experiment, one other possible disturbing factor may receive mention. On the ordinary oat field the plants are all of the same age and approximately in the same condition throughout their growth, while in the experiment four large plots in different stages were present. In the latter case therefore the flies were—in theory at all events—given the choice of oats in all conditions and so might have been able to select only those in the most suitable state for oviposition, neglecting others which, though capable of sustaining the larvae, were nevertheless less attractive. This possibility cannot be entirely disposed of, since the oat when in flower, for instance, may emit some very attractive scent which would cause flies to desert plots not in flower. The experimenters do not consider, however, that this factor of choice was operative to any extent and the final view is expressed that the normal period of susceptibility in the oat grain begins very shortly before fertilisation and closes before the grain reaches half its ultimate size, with the probability that maximum susceptibility occurs at the time of fertilisation.

B. *Variety Trials.* Concurrently with the experiments just recorded, a series of variety trials was carried out which may be discussed somewhat briefly. Observations and experiments carried out in 1918 and previous years indicated that if several varieties of oat were sown on neighbouring plots on the same date, great variation could be detected in the extent to which the several kinds suffered from the attack of Frit. As the discovery of a resistant oat appeared to be one of the most likely means of solving the principal problem, considerable attention was given to the subject, but progress was found to be slow. Varieties which in one locality and one season gave promising results appeared to fail in another locality and another season, while, further, there was found to be a very real difficulty in estimating scientifically the infestation by

[1] Reproduced from Miscellaneous Publication No. 33 of the Ministry of Agriculture and Fisheries.

Frit Fly of the young plant. Varieties of oats have different habits of growth and react differently when attacked by the fly—some for instance appear very susceptible but recover well, while others are less susceptible but have little power of recovery. In the case of a variety trial, therefore, it is difficult to obtain any standard of comparison which is reliable and it was intended to hold over the following results until the precise reaction of oat varieties to the fly had been worked out in detail. As, however, other investigators are publishing results of variety trials, it seems well to follow suit, with the warning however that in the opinion of the investigators, the method of conducting the trials was not such as to give results of real accuracy.

The experiment was as follows: 13 varieties were sown on April 25th by means of a hand drill in narrow strips running the entire length of the field. On June 18th, the plots were sampled according to the method described on p. 454 except that owing to the plots being narrow but half the number of plants were taken. These sample plants were examined in the laboratory and were classified into: plants not attacked; plants attacked but sending up a panicle; plants attacked and showing no signs of "earing" and therefore more damaged than those in the previous category. The result was as follows:

	Plants not attacked	Attacked, but sending ear	Attacked, not sending ear	Relative numbers of plants in sample
1. Thousand Dollar	95·6	—	4·4	93
2. Supreme	91·3	2·1	6·6	94
3. Victory	83·3	5·1	11·6	62
4. Record	86·8	—	13·2	70
5. Yielder	77·4	2·6	20·0	87
6. Banner	75·3	1·0	23·7	100
7. Golden Rain	63·9	7·4	28·7	97
8. Black Tartarian	66·7	3·3	30·0	62
9. Abundance	56·6	10·9	32·5	86
10. Sandy	50·0	10·6	39·4	79
11. Leader	43·7	6·3	50·0	50
12. White Tartarian	39·4	10·6	50·0	68
13. Potato	6·1	7·7	86·2	67

In this list the varieties are arranged according to the third column—Thousand Dollar, which showed the smallest percentage of plants seriously attacked, being placed first. An alternative order of merit can be obtained if column 1—percentage of plants not attacked—is taken, but it will be seen that very little alteration in the order is caused—Record changing places with Victory and Black Tartarian with Golden

Rain. No further comment is needed except to point out that the numbers represent only the plants remaining on June 18th, no account being taken of plants which from Frit attack or other reasons had died out and disappeared previously. This dying out of plants is a difficult factor to deal with in a field experiment but it is one to which further attention should be given. So far as our observations go there is in practically every case a progressive loss of plants during the first six weeks after the crop appears; it may be due to frit, to wireworm, or other pests, and even perhaps to inclement weather conditions. The essential fact, however, is that the loss is not uniform in regard to a series of varieties and where the results of an experiment are based upon yield, entirely erroneous conclusions may be drawn. In order to indicate how considerable may be the effect, a fourth column on the preceding table indicates the relative numbers of plants in the samples—the variety with the highest number being rated at 100. Of course, neither the sowing nor sampling was sufficiently uniform to justify the expectation of more than approximately the same number of plants in each sample, but the difference is so great that it cannot be due to experimental errors of this description.

After sampling on June 18th, the variety plots were left until harvest when they were again sampled and the spikelets analysed for the second brood attack, three categories being recognised: sound grain, grain definitely attacked by Frit, and blind spikelets. The results were as follows:

Variety	% frit	% blind	% sound	Order in Spring attack	1922 % frit
1. Golden Rain	7·7	5·3	87·0	7	—
2. Victory	9·3	14·0	76·7	3	16·5
3. Yielder	9·3	11·8	78·9	5	17·6
4. Black Tartarian	9·5	6·4	84·1	8	21·6
5. Thousand Dollar	10·2	2·2	87·6	1	—
6. Supreme	10·3	13·7	76·0	2	20·7
7. Banner	11·0	8·0	81·0	6	—
8. Record	14·4	16·3	69·3	4	—
9. Leader	16·7	12·2	71·1	11	22·4
10. Sandy	17·1	11·5	71·4	10	—
11. Abundance	18·2	10·9	70·9	9	24·0
12. Potato	21·2	17·6	61·2	13	15·5
13. White Tartarian	21·7	12·8	65·5	12	—

In column 4 of the above table is given the position of the variety in regard to the spring attack as indicated in the table on p. 462. It is evident that, judged by the percentage of frit-destroyed grains, there is

extraordinarily little correlation between the respective resistance to the spring attack and to the grain attack, suggesting that early maturity does not necessarily follow upon rapid growth in the plants' earlier stages. Also, the percentage of sterile spikelets appears to have no correlation with either of the Frit attacks and on this particular occasion it is difficult to believe that this pest was in any way concerned in the production of blindness.

Finally, reference may be made to a fifth column in the table which gives the percentage of grains attacked by Frit in a small variety trial carried out at Harpenden in 1922. All the varieties tested at Long Sutton were not grown at Harpenden, but it is of interest to note that, with one exception, where the same varieties were grown, each retains the same position in the list. For the behaviour of Potato, the most attacked variety at Long Sutton and the least at Harpenden, no explanation can be offered. At this point this series of trials may be left as the results are not considered to be sufficiently accurate to justify any elaborate deductions. The difficulty in obtaining in variety trials such as those described any results of a known order of accuracy appeared so great that it was decided to abandon field trials and to attempt on a small scale to discover the fundamentals concerned in the reaction of the young oat plant to Frit attack. A succeeding paper will deal with such an attempt and also with certain experiments to determine the exact period of susceptibility of the oat plant to the spring attack.

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OSCINELLA FRIT, LINN. AN INVESTIGATION TO DETERMINE HOW FAR VARIETAL DIFFERENCES MAY INFLUENCE INFESTATION OF THE OAT PLANT

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I. Introductory Remarks.

Although it is realised that a number of different workers have been engaged on the problem of varietal resistance, little appears to have been published on this subject and the only papers which have been consulted are those by Harper Gray (2), Fryer (1), Kleine (3) and Petherbridge (4). Kleine's records fail to distinguish adequately between the large number of varieties used, while both Harper Gray and Fryer base their conclusions on the percentage of damaged *plants*, or some variation of this method of evaluation. As yield is dependent on the number of stems capable of producing panicles as well as on the extent of the grain infestation, this method of differentiating between varieties is not effective unless the infestation varies between very wide limits. Taking an

extreme case, two varieties may show 30 per cent. and 60 per cent. unattacked plants respectively. If the plants in each variety are capable of producing four stems, the unattacked stems in the first case will range from 30–82·5 per cent. and in the second case from 60–90 per cent. Thus there may be the same total production of effective stems although the unattacked plants differ by 30 per cent. in the two cases. No reliable data are available to enable a comparison to be made between the many different varieties of oat in relation to infestation by *O. frit*. A series of investigations, of which the following study is a member, has been planned to determine (1) whether varietal differences influence infestation under similar environmental conditions, (2) how varieties react to varying environmental conditions, and (3) the factors controlling the variations in intensity of infestation, particularly when the limits of the variation are sufficiently wide to be of practical importance.

It is not claimed that the results recorded here are in any way final, because other variations in environment may react on these varieties of the oat quite differently, producing slight physico-chemical or physiological changes which even though only slight may be sufficient to alter susceptibility very materially, probably through the operation of a time factor. However this may be, it is suggested that only by means of detailed and statistical analyses can reliable conservative conclusions be drawn from the results of experiments of this nature. Such trials whether designed as frit fly investigations or for more general purposes usually show different results on the part of the varieties concerned. It is then often forgotten that although the facts observed may be true under the conditions of the experiment yet no generalisation is permissible unless the experimental errors can be defined, for frequently these errors are so large that observed differences, apparently considerable in themselves, may not be significant.

Finally, it should be recorded that the statistical analyses were made at Oxford, while Mr Gibson (now of the Scilly Islands Experimental Station) carried out most of the practical work at Harpenden.

Our sincere thanks are due to Mr R. A. Fisher, Statistician, of Rothamsted, who has kindly looked through this paper in MSS. and made valuable suggestions.

II. Method of Procedure.

From previous experience it was concluded that adequate analyses could be made on small plots only, the stem analysis having to be conducted in the shortest possible time. Even on plots of the small size

used during this investigation it was noted that environmental conditions were not absolutely uniform. To acquire preliminary data as to the effect of the environmental factors, the investigation was conducted in duplicate in widely separate localities, one set of experiments being arranged in the garden of the Pathological Laboratory at Harpenden, Hertfordshire, and the other on the University Farm, Sandford-on-Thames, Oxon., the experiments being identical except for locality. On plots running north and south and measuring 27 feet in length, fourteen drills 1½″ deep were made at distances of 9 inches. In these drills the twelve varieties recorded below were sown on 18. iv. 23, as evenly as possible, at the rate of 1¼ ounces of seed per 27 feet (which was calculated to be a mean field rate), the varietal order, which was the same in each series, being *Supreme*, *Victory*, *Record*, *Leader*, *Black Tartarian*, *Yielder*, *Goldfinder*, *Captain*, *Sir D. Haig*, *Superb*, *New Abundance* and *Scotch Potato*. The outside drills (*Abundance*) merely maintained normal conditions for the adjacent rows. Wire netting served to prevent rabbit and bird damage.

Our thanks are due to Messrs Garton, Ltd., Warrington, for their courtesy in supplying the necessary seed.

The following prearranged scheme of observations was followed where practicable during the growing season:

(1) the plants in the drills were counted on 18. v. 23;

(2) samples of each variety were pressed weekly for reference, each sample consisting of four plants, two of the mean type and one each of the mean extreme types;

(3) the rate of panicle shooting was observed;

(4) the dates of flowering (date pollen was ready to shed on the upper spikelets) were recorded;

(5) the dates when the seeds of the upper spikelets reached the "milky" stage were recorded;

(6) sampling for estimation of extent of infestation was conducted as follows:

(*a*) *Stem.* Strings were stretched across the drills at intervals of 4 feet and all the plants collected in that portion of each drill 4 inches on each side of the string. Thus for each variety about one-sixth of the total number of plants were available for analysis. The sample plants were carefully stripped and each stem, from the buds upwards, examined under the binocular microscope for evidence of frit infestation. Samples were collected on a prearranged date, 25. vi. 23, after which it was considered that newly-formed tillers would fail to produce panicles of importance in relation to yield[1]. It was found impossible in many cases

[1] See p. 469.

to identify correctly the different stems of the plant and a rough classification of stems was adopted at Oxford, based on the length of the stem including the blade, the four classes used being buds, 1–6″, 6–12″, and 12″ and over. Stems below 1″ in length were usually covered by the sheaths and were included with the buds.

(*b*) *Grain.* At Oxford the grain was sampled by collecting separately the panicles from the seven remaining sections when the seed had matured. For examination the panicles from each section were stripped, the spikelets well mixed and a number of spikelets from the centre of the small heap examined for infestation, the main and bosom grains being examined separately. At Harpenden the grain of each variety was collected in bulk. The panicles were stripped, the grain freed from the glumes, mixed, spread in layers one grain deep and sampled six times mechanically and regularly. In the case of the grain a certain difficulty presented itself, inasmuch as some ovules and seeds do not develop. Lack of development of the ovule may be due either to failure in the process of fertilisation or to the attack of the frit fly or other pest. Very immature seeds, as the result of infestation, may cease growth immediately after fertilisation, in which case the small fly larva may die or migrate leaving very little evidence of its work. As it was frequently impossible to determine exactly the cause of this "blindness" all doubtful cases have been included with the clean undeveloped grain under the heading of "blind" grain.

III. Experimental Data.

(*a*) *Stem.*

The minimum, mean and maximum numbers of plants per drill at Oxford on 18. v. 23 were 292, 446 and 598 respectively, the range being higher at Harpenden where conditions were better. The minimum, mean and maximum numbers of plants and stems in the samples taken on June 25 were (*a*) Oxford, 43, 63, 98 and 126, 172, 225, and (*b*) Harpenden, 78, 87, 156 and 307, 413, 741 respectively. Thus the plants at Harpenden produced 1·74 times the mean number of stems produced by the plants at Oxford. The mean infestations shown by the series of samples across the drills, the varieties being taken collectively, were (*a*) Oxford, 61·1, 56·4, 62·9, 56·4, 58·3, 56·4 per cent., and (*b*) Harpenden, 53·8, 62·3, 65·1, 67·9, 68·8 and 70·4 per cent. In the latter locality, the decreasing infestation was in the direction of the prevailing wind.

In Table I are shown the arithmetic mean percentage infestations of stems and grain for the twelve varieties of oats under observation at

Harpenden and Oxford, together with the standard errors of the means as determined from the formula $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, where d is the difference between the observed value and the arithmetic mean of the series, the number of observations (n) being small. The exposed stems only are included in these means, the small hidden tillers (buds) being omitted[1], and the records are set out in the plot order.

Table I.

Arithmetic mean percentage of infestation with standard error.

	O = Oxford	H = Harpenden	
Name of variety		Stem (omitting buds)	Grain
Supreme	O	64·4±2·9	42·4±2·9
	H	71·1±2·7	16·0±0·7
Victory	O	58·0±2·0	33·6±1·8
	H	63·4±2·4	17·0±0·5
Record	O	58·2±6·8	34·7±2·7
	H	71·0±2·4	13·9±0·8
Leader	O	57·7±4·9	37·8±2·5
	H	66·9±2·3	15·9±1·6
Black Tartarian	O	65·8±4·1	35·1±1·8
	H	63·6±2·6	20·7±0·8
Yielder	O	65·2±3·3	42·1±3·3
	H	66·6±3·3	16·0±1·8
Goldfinder	O	46·1±3·7	36·7±1·5
	H	56·5±6·7	15·6±0·7
Captain	O	60·3±3·8	38·1±4·0
	H	66·0±2·8	15·5±0·6
Sir D. Haig	O	54·4±6·0	32·2±2·7
	H	63·7±4·9	22·4±1·3
Superb	O	68·3±5·7	38·0±1·7
	H	68·1±2·8	13·7±0·9
New Abundance	O	48·3±6·0	34·9±2·6
	H	58·0±4·3	28·9±1·6
Scotch Potato	O	56·0±5·1	26·7±2·4
	H	61·9±3·7	13·4±1·1

The sampling date for the stem infestation analysis was arranged originally for 7. vi. 23, but it was observed at Harpenden that eggs were not visible on the oat stems until the end of May. In consequence of this observation it was thought that the main attack had not occurred in that place and therefore the later date (25. vi. 23) was arranged. It was discovered afterwards that eggs were deposited below the surface of the soil. The sampling prearranged for 7. vi. 23, when it was possible to distinguish between main stems and tillers, having been conducted at Oxford, the results are recorded in Table II. In this table are shown the

[1] See pp. 468 and 470.

percentage infestations of main stems, exposed tillers, total stems omitting hidden tillers, together with the records of the later sampling for comparison. In each case the percentage was calculated from the samples treated as a whole.

Table II.

Percentage infestation of stem, Oxford.

Variety	Main stems	Exposed tillers	Sampled on 7. vi. 23 Total stems	Sampled on 25. vi. 23 Total stems
Supreme	57·0	55·4	56·1	64·2
Victory	59·8	30·7	40·2	59·1
Record	39·3	39·4	39·4	58·7
Leader	63·0	52·1	56·5	61·9
Black Tartarian	53·3	53·3	53·3	64·1
Yielder	60·1	46·3	51·5	64·4
Goldfinder	33·0	41·6	37·2	46·2
Captain	61·2	50·0	56·2	60·3
Sir D. Haig	51·1	54·3	53·0	54·8
Superb	53·3	31·4	43·4	69·9
New Abundance	37·5	50·0	44·0	49·3
Scotch Potato	53·8	49·3	51·3	58·8
Mean percentage	51·9	46·1	48·5	59·6

The mean percentage infestations for main and exposed tillers were 51·9 and 46·1 respectively, the closeness of the values showing that the fly quickly deserted the main stems in favour of the young tillers for oviposition purposes. The increase in the mean infestation of total stems as the result of exposure to infection for 18 days following 7. vi. 23 was only 11·1 per cent.[1] The minimum, mean and maximum records for the hidden tiller infestation were (*a*) June 7, 8·2, 14·9, 21·2 per cent., (*b*) June 25, 6·5, 25·8 and 37·9 per cent. respectively. The range of attack for each size class of stem is shown in Table III, the observations having been made on the later sample.

Table III.

Percentage infestation of stem, sample 25. vi. 23, *Oxford.*

(*a*) Attack including growing point.
(*b*) Attack excluding same.

Length of stem including blades		Minimum	Mean	Maximum
Buds plus hidden tillers		6·5	25·8	37·9
1–6″	(*a*)	58·1	69·9	78·0
	(*b*)	0	1·6	4·7
6–12″	(*a*)	54·7	65·2	80·0
	(*b*)	0	2·3	7·9
12″ and over	(*a*)	11·8	19·9	30·0
	(*b*)	0	2·6	8·8

[1] A mean increase of 17 per cent. in the total number of exposed stems, available for the fly, was observed by the date of the second sampling.

The attack on exposed stems below 12″ in height, including the longest blade, was three times as intense as that on those above 12″. Of the latter, one-fifth were rendered incapable of producing panicles, while one-quarter of the buds were destroyed, the latter damage being of no economic importance except in so far as it increases the July population of flies.

Comparing the different varieties the minimum, mean and maximum percentages of the stems 12″ and over in length were 10·3, 17·6 and 24·8 and of the buds 16·1, 22·9 and 28·2. The effective attack on the most vigorous stems ranged from 11·8–30·0 per cent. but in relation to the total numbers of stems the range was only 2·0 1·5 per cent.; similarly, in the case of buds, the range of attack was 1·3–9·3 per cent.

It is stated frequently that different varieties of oat have different powers of recovery from infestation, by which is meant presumably that certain varieties yield better than others even though infestation may be apparently evenly distributed. This elusive quality, about which nothing is known, would seem to be dependent (1) on the attack away from the growing point, such stems being still capable of producing panicles, (2) on the early damage to hidden buds and small tillers, on which subsequent tillering depends, and (3) mainly on the capability of the plant to produce effective panicles after the first shoots have been destroyed. The first type of damage has been shown to be negligible (Table III (*b*)), while the second type was variable. Normally the attack on the buds and hidden tillers would have no effect on the yield of the crop, but variation in this type of damage (if early) might be of some importance.

The whole problem of recovery thus remains one of the many problems requiring elucidation.

(*b*) *Grain.*

Shooting of Panicles. The emergence of the panicles from the sheaths was spread over the month of July at Oxford. By July 8 the mean emergence was 8·8 per cent., *Record*, *Black Tartarian*, *Goldfinder* and *Scotch Potato* oats not having started, while *Captain* oats had reached 23·9 per cent. By July 16 the mean emergence was 59 per cent., *Leader* oat having completed emergence while *Sir Douglas Haig* and *Scotch Potato* oats were still over 20 per cent. below the mean. By July 24 *Sir D. Haig* oat was still 15 per cent. below the mean of 75·8 per cent., the remaining varieties closely approximating to the mean.

Although there was great variation in the time of emergence of the

panicles, the coefficient of correlation between the percentage emergence by July 16 and the percentage infestation of the grain was only $+ 0{\cdot}47 \pm 0{\cdot}15$; with the arithmetic mean percentage infestation of stem the coefficient was $+ 0{\cdot}28 \pm 0{\cdot}18$, showing that the rate of panicle shooting was dependent on varietal differences rather than on intensity of infestation of stem.

The minimum, mean and maximum numbers of seed examined were (*a*) Oxford 560, 665, 721, and (*b*) Harpenden 655, 802 and 935 respectively. The range of variation among the varieties of oat as determined at Oxford is shown in Table IV.

Table IV.

Percentage infestation and "blindness" of grain, Oxford.

	Minimum	Mean	Maximum
Percentage of Bosom Grain	18·5	44·1	50·9
Main Grain, infested	28·3	45·9	55·3
„ "blind"	3·2	7·5	14·3
Bosom Grain, infested	15·1	22·8	30·0
„ "blind"	7·3	13·3	30·7
Total grain, infested + "blind"	31·3	45·6	57·6
"Blind" grain	4·7	9·8	16·6
"Blind" grain, Harpenden	5·2	7·4	8·7

Scotch Potato oat with only 18·5 per cent. of bosom grain was far below the mean. The mean infestation of the main grain was 45·9 per cent. against 22·8 per cent. for the bosom grain, showing that the time factor was of considerable importance here. *Black Tartarian* oat produced the minimum quantity of "blind[1]" main grains (3·7 per cent.), *Abundance* oat producing the maximum quantity (14·3 per cent.). The varieties *Yielder* (30·7 per cent.) and *Captain* (29·3 per cent.) produced "blind" bosom grain in abnormal quantities, *Abundance* being next with 16·4 per cent., and the remainder ranging from 7–11 per cent. only. The mean total production of "blind" grain was considerable, being 9·8 and 7·4 per cent. at Oxford and Harpenden respectively.

In Table I are recorded the arithmetic mean percentage infestations of the grain for the twelve varieties of oats under observation at Oxford and Harpenden, together with the standard errors of the means. It should be noted that, owing to the different methods of sampling, the standard errors of the Oxford means are due to field variations as well as actual errors in sampling, while those of the Harpenden means are due to errors of sampling from a mass of material only.

[1] As indicated previously, "blindness" in the sense in which the term is used here means that the causal agent was unknown, but was considered not to be the frit fly.

IV. Statistical Analysis of Data.

(*a*) *Coefficients of correlation.*

The mutual relationship between two series of variates is best expressed by the coefficient of correlation (r), which may be determined readily from the formula $r = \frac{\Sigma(d_x d_y)}{n\sigma_x\sigma_y}$, the probable error of r being $\frac{0{\cdot}674(1-r^2)}{\sqrt{n}}$. When r equals or exceeds six times the probable error, correlation between the variates definitely exists and in such cases if r is above 0·5 there is decided correlation. Some of the coefficients of correlation which have been calculated from the experimental data available are recorded in Table V.

Table V.

Coefficients of correlation.

A. *Stem.*	
Arithmetic mean percentage infestation, second sample, Oxford, with	
1. Percentage of stunted plants (6″ and less) in samples, Oxford	+0·69±0·10
2. Stem infestation, Harpenden	+0·75±0·08
3. Tillering capacity, Oxford	+0·41±0·16
4. Total stems per plant, Oxford	+0·42±0·16
5. 12″ stems and over per 100 plants, Oxford	−0·67±0·10
6. 6″ „ „ „ „	−0·38±0·17
7. Rate of panicle shooting...	+0·28±0·18
B. *Grain.*	
(*a*) Arithmetic mean percentage infestation, Oxford, with	
1. Stem infestation, Oxford	+0·43±0·16
2. "Blind" grain, Oxford	+0·56±0·13
3. Grain infestation, Harpenden	−0·07±0·19
4. Rate of panicle shooting...	+0·47±0·15
(*b*) Arithmetic mean percentage infestation, Harpenden, with	
1. Stem infestation, Harpenden	−0·50±0·14
2. "Blind" grain, Harpenden	+0·11±0·19
C.	
1. Infested plus "blind" grain, Oxford with Harpenden ...	+0·17±0·14
2. Varietal yield values, Oxford with Harpenden,	
(*x*) Infested grain only	+0·67±0·10
(*y*) Infested plus "blind" grain	+0·62±0·12

There was a decided correlation between infestation and stunting of the plant, but half the stunting was caused by other factors, *i.e.* our knowledge of the percentage infestation only gives as much information about the percentage of plants stunted as all other independent factors put together. Tillering capacity was not definitely correlated with infestation. The percentage increase between June 7 and June 25 in the

number of exposed tillers ranged from 6–124 with a mean of 44, but this additional tillering was not correlated either with main stem infestation ($r = -0{\cdot}006 \pm 0{\cdot}21$) or with exposed stem infestation definitely

$$(r = +0{\cdot}44 \pm 0{\cdot}17),$$

as determined on June 7. On the other hand, it is a matter of observation that a plant losing the main stem early shows advanced tillering in many cases. The sap supply to the damaged stem must be reduced considerably, although not entirely inhibited, the greater part of the material being available elsewhere. The production of stems 12″ and over in height showed a decided negative correlation with infestation, as was to be expected. Correlation between the stem infestations at Oxford and Harpenden was very high ($+0{\cdot}75 \pm 0{\cdot}08$), showing that variation in locality alone should not markedly affect the relative variations in stem infestation.

The position with regard to the grain was very different. The grain and stem infestations in the one locality showed no certain correlation, while the grain infestations at Oxford and Harpenden showed no correlation whatever ($-0{\cdot}068 \pm 0{\cdot}19$). The independence of the intensities of infestation of the stem and grain indicates that the grain infestation is very dependent on a time factor. As the production of "blind" grain was not correlated with infestation, it would appear that very few errors from this source were included with the estimates of frit infestation.

(*b*) *Varietal differences in intensity of infestation.*

Certain differences in intensity of infestation between the varieties of oats used during this investigation have been recorded for both stem and grain. The value of records of this type is greatly increased if they are subjected to statistical analysis (1) to determine whether any two values are significantly different or whether the observed differences may be ascribed to fluctuations of sampling, and (2) to combine the evidence derived from independent experiments. A short description of the formulae utilised may be useful to those unacquainted with statistical methods as they are generally applicable and are valuable aids in deducing conservative conclusions.

To determine whether two values x and y of arithmetic mean percentage infestation in the same series are significantly different (*i.e.* whether the observed difference in infestation is independent of fluctuations of sampling) it is necessary to divide the observed difference (D) between the arithmetic mean percentage infestations by the standard

error of the difference ϵ_{xy} obtained from the formula $\epsilon_{xy}^2 = \frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}$, where σ^2 equals $\frac{\Sigma d^2}{n-1}$. If $\frac{D}{\epsilon_{xy}}$ equals or exceeds 2·57, then the chances are 100 to 1 or more that the observed difference is significant and not due to fluctuations of sampling.

The values obtained independently at Oxford and Harpenden have been combined[1] by means of the corresponding standard errors from the formula

$$X = \frac{\frac{D_O}{\epsilon_O^2} + \frac{D_H}{\epsilon_H^2}}{\frac{1}{\epsilon_O^2} + \frac{1}{\epsilon_H^2}},$$

where X is the mean precentage difference in infestation between two varieties weighted according to the independent observations, D the

Table VI.

Stem analysis.

O = Oxford H = Harpenden

Variety		Difference between arithmetic mean percentage infestations (D)	Standard error of $D = \epsilon_{xy}$	$\frac{D}{\epsilon_{xy}}$	Weighted mean difference in percentage infestation (X)	Standard error (E) of X	$\frac{X}{E}$
Supreme	O	18·3	4·7	3·88	17·2	3·9	4·36
	H	14·6	7·2	2·02			
Superb	O	22·2	6·8	3·28	17·2	5·0	3·46
	H	11·6	7·2	1·60			
Yielder	O	19·1	4·9	3·87	16·4	4·1	3·99
	H	10·1	7·5	1·36			
Black Tartarian	O	19·7	5·5	3·58	15·1	4·4	3·46
	H	7·1	7·2	1·00			
Captain	O	14·2	5·3	2·67	12·9	4·3	3·01
	H	9·5	7·2	1·31			
Victory	O	11·9	4·2	2·83	10·5	3·6	2·90
	H	6·9	7·1	0·97			
Record	O	12·1	7·8	1·56	13·4	5·2	2·56
	H	14·5	7·1	2·04			
Leader	O	11·6	6·1	1·90	11·1	4·6	2·41
	H	10·4	7·1	1·48			
Scotch Potato	O	9·9	6·4	1·55	8·0	4·9	1·63
	H	5·4	7·6	0·71			
Sir D. Haig	O	8·3	7·0	1·18	7·8	5·3	1·44
	H	7·2	8·3	0·87			
New Abundance	O	2·2	7·1	0·31	1·9	5·3	0·36
	H	1·5	7·9	0·19			
Goldfinder	Variety with the minimum arithmetic mean percentage infestation, with which the remainder have been compared.						

[1] We are indebted to Mr Fisher for this method of calculating the weighted mean.

Table VII.

Grain analysis.

O = Oxford H = Harpenden

Variety		Difference between arithmetic mean percentage infestations (D)	Standard error of $D = \epsilon_{xy}$	$\frac{D}{\epsilon_{xy}}$	Weighted mean difference in percentage infestation (X)	Standard error (E) of X	$\frac{X}{E}$
New Abundance	O	8·2	3·5	2·32	14·3	1·75	8·2
	H	15·5	2·0	7·92			
Sir D. Haig	O	5·5	3·6	1·53	8·3	1·54	5·4
	H	9·0	1·7	5·30			
Black Tartarian	O	8·4	2·9	2·86	7·5	1·22	6·3
	H	7·3	1·3	5·40			
Yielder	O	15·4	4·0	3·81	5·4	1·90	2·8
	H	2·6	2·1	1·21			
Victory	O	6·9	3·0	2·33	4·6	1·19	3·9
	H	3·6	1·2	2·98			
Leader	O	11·1	3·5	3·18	4·6	1·71	2·7
	H	2·5	2·0	1·28			
Supreme	O	15·7	3·7	4·20	4·0	1·23	3·3
	H	2·6	1·3	2·00			
Goldfinder	O	10·0	2·8	3·68	3·6	1·17	3·0
	H	2·2	1·3	1·71			
Captain	O	11·4	4·7	2·44	2·7	1·19	2·3
	H	2·1	1·2	1·71			
Superb	O	11·3	2·9	3·85	2·4	1·29	1·9
	H	0·3	1·4	0·21			
Record	O	8·0	3·6	2·21	1·3	1·17	1·1
	H	0·5	1·2	0·40			
Potato	Variety with the minimum arithmetic mean percentage infestation, with which the remainder have been compared.						

observed difference between the arithmetic mean percentage infestations, and ϵ the standard error, denoted above as ϵ_{xy}. The standard error (E) of X is $\frac{1}{\sqrt{1/\epsilon_O^2 + 1/\epsilon_H^2}}$. The value of the weighted mean difference in mean percentage infestation between two varieties may be tested by the relation of X to E as indicated above.

The results of such analyses for both stem and grain infestations are shown in Tables VI and VII and the importance of this method of examining the results of observation is exemplified by the two cases of grain infestation, *Leader* and *Captain*, at Oxford. In addition two independent values, each of doubtful significance alone, may reinforce one another, giving a significant weighted mean value, *e.g.* Table VI, *Record*.

The coefficients of variability indicate that the variation between

parallel samples was distinctly lower in general at Harpenden than at Oxford, but *Goldfinder* at Harpenden was exceptional in this respect, thereby increasing the Harpenden standard errors and somewhat reducing the weight of the Harpenden data in the weighted means for the stem infestation.

V. Interpretation of Data.

Taking *Supreme* oats as an example to explain the meaning of the values recorded in Tables VI and VII, the weighted mean difference in percentage infestation of stem from the value observed for *Goldfinder* oats was $17{\cdot}2 \pm 3{\cdot}9$. The figure 17·2 is the *most probable value* of the difference and is assumed to be the true value under the varied conditions of both series of experiments. Both + and − deviations are equally likely to occur in other series of experiments, but less and less frequently as they increase in magnitude, while the chances are about 330 to 1 that most of the deviations will lie within the limits of the mean ± three times the standard error.

Stem. *Supreme*, *Superb*, *Yielder*, *Black Tartarian*, *Captain* and *Victory* oats, in this order, suffered from 17·2–10·5 per cent. greater stem infestation than *Scotch Potato*, *Sir Douglas Haig*, *New Abundance* and *Goldfinder* oats. The latter form a group in which the differences were not significant under the conditions of these experiments. *Record* oat should probably be included with the first series, while the position of *Leader* oat is doubtful[1].

With regard to the pressed samples of the several varieties, a careful examination has up to the present failed to show any differences in growth, tillering, etc. which can be correlated with the observed differences in extent of infestation, even in the cases of the extremes. At the same time, this line of investigation has not been carried as far as is desirable and as additional material accumulates, the above observation may have to be modified.

Grain. With regard to the grain infestation the group suffering least infestation included *Captain*, *Superb*, *Record* and *Scotch Potato* oats. *Victory*, *Leader*, *Supreme* and *Goldfinder* oats suffered less than 5 per cent. heavier infestation; *Sir Douglas Haig*, *Black Tartarian* and *Yielder* oats less than 10 per cent. but more than 5 per cent. heavier infestation; while

[1] Mr Fisher has calculated the weighted average stem infestations, comparing the varieties with the mean infestation of 62·45 per cent., with the result that *Supreme* (68·62 per cent.) and *Superb* (68·17 per cent.) are again shown to be significantly highly infested and *New Abundance* (54·41 per cent.) and *Goldfinder* (52·65 per cent.) significantly low.

New Abundance was decidedly the heaviest sufferer, showing an infestation 14·3 per cent. above that of *Scotch Potato*.

The range of the differences was of the same order in both stem and grain infestations.

Comparative Yield Values. Yield of straw is dependent on (1) the percentage of uninfested stems, and (2) the tillering capacity of the plants, particularly when subjected to infestation. No observations have been made in relation to this subject.

Yield of grain is dependent on (1) the percentage of clean stems, (2) the percentage of clean seeds borne by the clean stems, (3) the extent of material damage to the seed, which will probably be a more or less constant factor for all varieties, and (4) the average size and weight of the clean seed of the variety. Therefore by taking both stem and grain infestations into consideration values representing percentage yield of unattacked grain may be calculated and compared. These values are shown in Table VIII, column A being based on the percentage of grain free from frit infestation and column B on the percentage of grain free from both frit infestation and blindness. Thus assuming the maximum yield to have been 100 seeds, Table VIII shows the relative number of clean seeds produced by each variety under the conditions of these experiments. The Oxford and Harpenden values thus obtained have a coefficient of correlation of $+ 0{\cdot}67 \pm 0{\cdot}10$.

Table VIII.

Comparative Varietal Yield Values.

A. Based on percentage of frit-free grain.

B. Based on percentage of unattacked developed grain*.

Variety	Oxford		Harpenden	
	A	B	A	B
Goldfinder	34·2±2·5	29·6	36·7±6·3	33·0
New Abundance	33·6±4·2	25·7	29·8±3·4	26·4
Scotch Potato	32·3±4·0	30·2	33·0±3·5	30·2
Sir D. Haig	30·8±4·3	27·4	28·1±4·2	25·3
Victory	27·8±1·6	24·4	30·3±2·2	27·9
Record	27·4±4·6	23·7	25·0±2·3	23·2
Leader	26·3±3·2	22·7	27·8±2·2	26·3
Captain	24·5±2·9	18·1	28·7±2·6	25·8
Black Tartarian	22·2±2·7	20·4	28·0±2·3	26·6
Supreme	20·5±2·0	17·6	24·3±2·5	22·1
Yielder	20·2±2·3	14·8	28·1±3·1	25·4
Superb	19·6±3·6	17·0	27·6±2·6	25·3

* The standard deviations of the mean values recorded in columns B have not been calculated, but will be of the same order as those of the respective A columns.

Tested by the relation of the percentage difference (D) to the standard deviation (ϵ_{xy}), none of the Harpenden percentage differences are significant among themselves, owing to the relatively great variability of the stem infestation in *Goldfinder* oat. Approximations to the standard deviations of the yield values have been calculated from the formula

$$\sigma^2_{\text{Stem}\times\text{Grain}} = M_S{}^2\sigma_G{}^2 + M_G{}^2\sigma_S{}^2,$$

and weighted mean yield values then obtained by the method previously indicated, these values being recorded in Table IX.

Table IX.

Analysis of yield of frit-free grain.

Variety	Weighted mean difference in percentage yields	$\frac{X}{E}$
Goldfinder	Variety with maximum yield value, with which the remainder have been compared	
Scotch Potato	$-2{\cdot}4\pm3{\cdot}9$	0·62
New Abundance	$-2{\cdot}5\pm4{\cdot}1$	0·62
Sir Douglas Haig	$-4{\cdot}9\pm4{\cdot}1$	1·19
Victory	$-6{\cdot}4\pm2{\cdot}7$	2·40
Leader	$-8{\cdot}0\pm3{\cdot}4$	2·35
Record	$-8{\cdot}7\pm4{\cdot}1$	2·13
Captain	$-9{\cdot}3\pm3{\cdot}3$	2·80
Black Tartarian	$-11{\cdot}4\pm3{\cdot}2$	3·55
Yielder	$-13{\cdot}1\pm3{\cdot}0$	4·32
Superb	$-13{\cdot}1\pm3{\cdot}6$	3·59
Supreme	$-13{\cdot}4\pm3{\cdot}2$	3·55

The varieties *Goldfinder*, *Scotch Potato*, *New Abundance*, *Sir Douglas Haig*, *Victory*, *Leader* and *Record* form a group in which the observed differences in percentage yield of frit-free grain may be ascribed to fluctuations of sampling. *Captain*, *Black Tartarian*, *Yielder*, *Superb* and *Supreme* oats showed significant negative differences, varying from 9·3–13·4 per cent. respectively, when compared with *Goldfinder* oat. The latter variety had a percentage yield value of unattacked developed grain equal to 35 per cent. only of the total yield possible under the conditions of the experiments, the observed differences between the two groups therefore being of importance.

VI. Summary.

Twelve varieties of oat (*Supreme*, *Victory*, *Record*, *Leader*, *Black Tartarian*, *Yielder*, *Goldfinder*, *Captain*, *Sir Douglas Haig*, *Superb*, *New Abundance* and *Scotch Potato*) have been grown in widely separated localities, namely, Sandford, Oxon., and Harpenden, Herts., where they

were subject to the attack of *Oscinella frit*, L. The infestations of stem and grain were observed in detail and the values obtained subjected to statistical analysis.

1. The stem infestation showed an increase of 11 per cent. only, 18 days after June 7.

2. The attack on stems 12″ in length (including blades) and over was only one-third as intense as that on the remaining stems; 20 per cent. of the former were rendered incapable of producing panicles; 25 per cent. of the hidden buds plus small tillers were destroyed. Infestation away from the growing point averaged 2 per cent.

3. The infestation of the main grain reached 45·9 per cent. against 22·8 per cent. for the bosom grain, at Oxford, indicating the importance of the time factor.

4. The production of "blind" glumes (*i.e.* those containing undeveloped seed, causal agent unknown) was considerable (7–9 per cent.), but independent of frit infestation of grain.

5. The correlation between stem infestations at Oxford and Harpenden was high, but the grain infestations showed no correlation whatever; neither was there any definite evidence of correlation between stem and grain infestations in either locality. No correlation between infestation and tillering capacity was discovered, but the production of vigorous stems was decidedly correlated with the absence of infestation.

6. Weighted mean differences in mean percentage infestations both for stem and grain are recorded for each variety, comparisons being made with the *Goldfinder* oat in the former case and the *Scotch Potato* oat in the latter case.

7. With regard to the stem infestation, *Scotch Potato*, *Sir Douglas Haig*, *New Abundance* and *Goldfinder* oats were proved, under the conditions of these experiments, to be least liable to infestation, but it was not possible to differentiate between them. The remaining varieties suffered from 10–17 per cent. heavier stem infestation, the differences observed being significant.

8. With regard to the grain infestation, *Captain*, *Superb*, *Record* and *Scotch Potato* oats suffered the least infestation, *New Abundance* oat being most heavily infested.

9. Considering the percentage yields of unattacked developed grain, *Captain*, *Black Tartarian*, *Yielder*, *Superb* and *Supreme* oats, when compared with *Goldfinder* oat, were found to yield less by 9·3–13·4 per cent., the yield of *Goldfinder* oat being equal to only 35 per cent. of the total yield possible under the conditions of this experiment.

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THE APPLE BUD WEEVIL, *ANTHONOMUS CINCTUS*, KOLL.

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(With Plate XVI and 10 Text-figures.)

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1. Introduction.

In the spring of 1921 complaints were received from Mr W. G. Kent, the Horticultural Superintendent of the Isle of Ely County Council, that apple fruit buds were being killed by a weevil very similar to the Apple Blossom weevil (*A. pomorum*). This damage was first noticed by Mr H. J. Gardner of Wisbech whilst pruning cordons in March. On April 8th we visited Messrs Gardner Bros. and brought back a few of the weevil larvae in the buds they had hollowed out.

From the resemblance of these to the larvae of *A. pomorum* we formed the opinion that the abnormally warm conditions prevailing in this district in the early part of 1921 had caused *A. pomorum* to lay their eggs very early; and, as no blossoms were available, we presumed they had laid them on the blossom buds. At the beginning of May the larvae

pupated and early in June weevils hatched out. These were identified by Messrs Champion and Harwood (P.) as *Anthonomus cinctus*, Koll. On August 14th, 1921, a single specimen of this weevil was found, in company with *A. pomorum*, under apple bark in an orchard adjoining Darenth Wood (in Kent) by Mr P. Harwood (1).

In the spring of 1922 injured fruit buds containing weevil larvae were found in two other portions of Messrs Gardners' orchards, situated more than 100 yards from the cordons where the weevils were found in 1921.

A search was made in the neighbourhood of Cambridge for buds containing this weevil and, on May 14th, 1922, we found three apple buds hollowed out, each containing a bud weevil larva, in an orchard near Histon. Young pear trees situated about 200 yards from the infected apples showed no sign of attack, although on the Continent this insect is regarded as a pear pest.

Later in the season, further evidence of this pest in another orchard (nearly two miles from the one previously mentioned) was afforded by the presence of dead hollowed-out buds with the characteristic exit hole of the weevil (see Plate XVI, fig. 3). On November 8th bud weevils were found on the "tanglefoot" bands placed round the trunks of these trees.

2. Synonyms.

Anthonomus cinctus Kollar, Redtb. Fauna 2. 309.
Anthonomus pyri Boh. Schonherr Gen. and Spec. Curculionidum 7. 2. 215.
Anthonomus bituberculatus Thoms. Skandinaviens Coleoptera, 1868.

This species should not be confused with *A. cinctus* Thoms. = *A. inversus* Bedel = *A. ulmi* Debr. (non Deg.) and British Catalogue. Von Frauenfeld, *Verh. z. b. Ges. Wien*, 1872, p. 393, describes under the name of *A. cinctus* Sch. a species which lives on *Pyrus salicifolius*. The identity of this insect has not been determined (Bedel) (2).

3. Description.

Adult (see Fig. 1).

Length (including rostrum) 4·5–5·0 mm.

Brownish-red or purplish-red, clothed with white, golden and black pubescence.

Head with two tufts of white pubescence between the large prominent eyes.

Rostrum long, slightly curved, rugose and ridged, apex and sides black.

Thorax with numerous coarse punctures, clothed with golden and

black pubescence, with a line of coarse white pubescence running down the centre.

Scutellum small, with dense coarse white pubescence.

Elytra covered with white, golden and black pubescence and having a marked transverse fascia of white pubescence behind the middle, broader at the margins, bordered in front and behind by black hairs; *bearing a tubercle near the base of the third interstice densely covered with black hairs* (in addition to the shoulder characteristic of Anthonomina). Interstices wide and rough. Striae coarsely punctured.

Legs reddish-brown with black patches in places but very variable. Anterior femora with a very strong tooth. Basal half of anterior tibiae strongly sinuate on inner margin, other tibiae almost straight, but bent at base.

External Sexual Differences. The sexes can be distinguished by examination of the ventral surfaces, without injury to the specimens. In the *male* the posterior margin of the pygidium is folded into a definite ridge forming an angle of about 90° at the apex, the angle increasing towards the base. The ventral side of the ridge is smooth and shining: the dorsal surface of the ridge is covered with white hairs. (Apart from the ridge the pygidium is markedly convex.) In the female the posterior margin of the pygidium is not folded, but is markedly convex.

There are also slight differences in the rostra. In the *male* the rostrum is shorter, narrower at the base, and gradually enlarging to the apex. In the *female* the rostrum is longer, thicker at the base and enlarging from the point of insertion of the antennae to the apex.

Plate XVI, fig. 2 shows the male genitalia, and may prove useful in distinguishing it from allied species.

Distinction from Allied Species.

A. cinctus resembles fairly closely some of the other species of *Anthonomus*. It most closely resembles *A. ulmi* and may be present in some collections under this name, as it runs down to this species in Fowler's key better than *A. ulmi* itself.

A. cinctus (Koll.) Larger	*A. ulmi* (De G.) Smaller
Reddish-brown with a purplish tint, darker than *A. ulmi*.	Brownish-red (very variable).
Tubercle near the base of the 3rd interstice of each elytron densely covered with black hair.	Small tubercles *sometimes* present near base of 3rd interstice of each elytron; fewer black hairs.
Rostrum longer, 1·4–1·6 mm.	Rostrum shorter, 1·0–1·35 mm.
Antennae longer, 1·95–2·05 mm.	Antennae shorter, 1·3–1·4 mm.
Thorax relatively wider, about 1·33–1·4 times the length.	Thorax relatively narrower, from about 1·2–1·3 times the length.

Fowler says of *A. ulmi*, De G. "Elytra with a little oblong tubercle or small prominence at the base of the third interstice," but he adds later, "the points of difference which have usually been given as separating it from the following (species) are sometimes hard to make out satisfactorily."

Reitter(3) says of *A. inversus*, Bedel (= *A. ulmi*, De G.), third interstice not broadened, rarely raised and with no black hairy protuberance.

A. pedicularius, L. can be distinguished from *A. cinctus* by the following characters. Absence of tubercle on 3rd interstice, a much shorter rostrum, much straighter and scarcely sinuate anterior tibiae, smaller tooth on anterior femora and in fresh specimens by the presence of two white fasciae on the elytra, the anterior of which is not so marked.

A. chevrolati, Desb. can be distinguished from *A. cinctus* by the absence of a tubercle on the third interstice, but right at the basal margin the extreme base of the third interstice is raised.

A. cinctus is easily distinguished from *A. pomorum* which is smaller, darker in colour, and has a strongly oblique white fascia on each elytron forming a V-shaped marking.

The Egg.

Time of Egg-laying. Most of the continental observers agree that eggs are laid in pear buds in the autumn. Boisduval(4) and Taschenberg(5) state that eggs are laid in the spring.

In 1922 none of our "sleeved" weevils laid eggs, but on February 3rd, 1923 we found eggs in apple buds near Histon, and were unable to find adults on or after this date. In 1923 all our "sleeved" weevils except two died without laying eggs. These two, one female and one male, were "re-sleeved" on October 24th, and on November 5th one egg was found. Another egg was laid between January 3rd and January 23rd, 1924. The male was dead on February 28th, and the female on March 6th.



Fig. 1. Egg of *A. cinctus*.

Position. The eggs are laid in the bud of the apple; and, in a very large percentage of cases, in the fruit buds. A hole is made in the bud by the female (see Plate XVI, fig. 3) usually just below or near the equator, and the egg is placed at the bottom of the burrow. The egg usually has two bud-scales immediately outside it, but it may be deeper, or underneath the outer bud-scale only. We never found more than one egg in a bud.

Structure. The egg measures 0·7–0·8 mm. in length, and 0·5–0·59 mm. in width. It is of a creamy-white colour, and smooth, without any distinctive markings. It is bean-shaped, being broader at the head end than at the distal end. Its shape is shown in Fig. 1.

Just before hatching the head darkens and can be seen through the transparent chitin; the proximal end of the egg for about two-thirds of



Fig. 2. *Anthonomus cinctus.* Third stage larva.

its length is more opaque than the rest, and white longitudinal marks (apparently the fat bodies) can be seen.

The distal end is somewhat transparent and dusky.

1*st Stage Larva.* Length in normal curled condition 0·9–1·0 mm.
Width of head 0·35 mm.
Spiracle. Length about 25μ, width 15μ.

This larva differs from the 3rd stage larva described below in the comparatively large size of the head, which is also lighter in colour. The mandibles have a distinct file-like serration on the lower edges of their two teeth, and the segments are very much more difficult to differentiate.

2nd Stage Larva. Length in normal curled condition about 1·8 mm.
Width of head 0·46 mm.
Spiracle. Length about 32 μ, width 22 μ.

This larva is intermediate in characteristics between the 1st and 3rd stages.

3rd Stage Larva. (See Fig. 2.) Length in normal curled position about 3·5 mm. (True length about 5·5 mm.)
Width of head ·55–·6 mm.

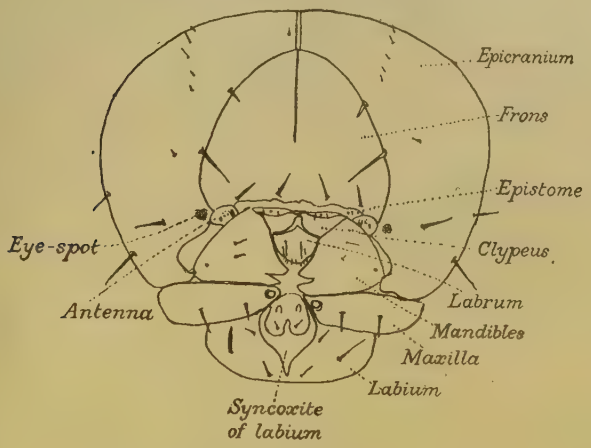


Fig. 3. Head of third stage larva of *A. cinctus*.

It resembles the larva of *A. pomorum* but is broader and more curled. The head is dark brown and fairly large. The body is creamy-white, soft and fleshy and with numerous transverse wrinkles. The skin is transparent and the outlines of the cream-coloured lobules of the fat bodies alternating with the darker colour of the body contents give the larva a characteristic appearance. The dorsal vessel is plainly visible as a brownish line down the centre of the dorsal surface and its pulsations can be seen under the lens.

The head (Fig. 3). The epicranium is divided down the middle by a suture and bears several longish setae, two pairs along the arms of the

epicranial suture and three pairs a little behind the anterior angles of the head. There are also five minute setae on either side of the vertex.

The sclerite formed of the fused frons and epistome is subcordate, with two setae on each side, one near the base of each antenna, and one on either side of the median line, together forming a transverse row at the anterior margin of the epistome. A marked sagittal furrow extends half-way from the vertex towards the anterior margin.

The anterior margin is thickened, and somewhat waved, and bears two pairs of small setae and two pits.

The clypeus and labrum together form a sub-triangular plate above the mandibles. The anterior border of the clypeus is emarginate. The labrum bears 16 setae pointing anteriorly and 2 pits. A conical projection from the labrum extends beneath the clypeus (see Fig. 4).

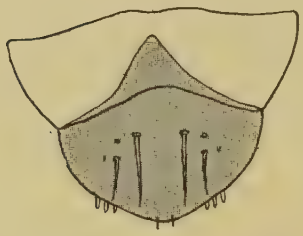


Fig. 4. Labium and clypeus of larva of *A. cinctus*.

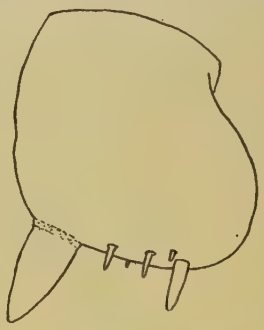


Fig. 5. Left antenna of third stage larva of *A. cinctus*.

The antennae (see Fig. 5) are pale-coloured and situated at the basal angle of the frons. Each is sub-globose, with a bullet-shaped process on the inner side and five smaller processes of similar shape on the outer side.

The *eyes* are represented on the inner margin of the epicranium by a dark pigmented spot just behind the base of the mandibles.

The *mandibles* (see Fig. 6) are stout with two bluntly-pointed teeth of equal size, dark ochreous-brown with the file-like serration on the inner edges of the teeth present in the other two stages, indistinct. Each bears two setae near the centre and a pit near the mandibular condyle.

The maxillae and labium (see Figs. 7 and 8) are prominent on the ventral surface. The maxillary cardo is small and has a bluntly-pointed process on the outer side of the base. The stipes is long, the surface of the

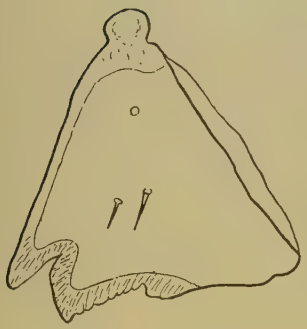


Fig. 6. Mandible of larva of *A. cinctus.*

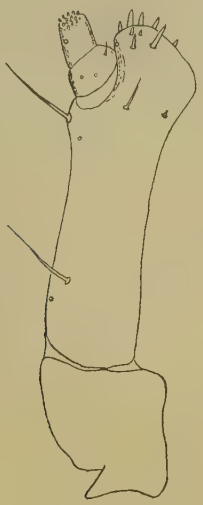


Fig. 7. Maxilla of larva of *A. cinctus.*

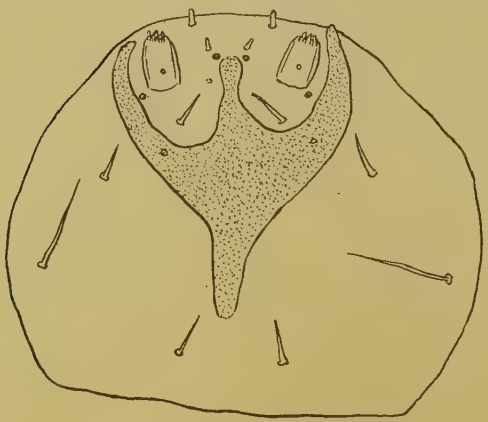


Fig. 8. Labium of larva of *A. cinctus.*

N.B. All these figures are drawn from a third stage larva.

mala bears a small sensory pit on its outer margin and nine hair-like papillae apically. The two-jointed palp has fifteen conical papillae on its disc-like apex and a small seta on its basal segment.

In addition there are on the stipes four setae, one external, close to the cardo, and two near the base of the palp, and one small one almost in line on the inner margin.

The base of the labium is composed of a fused membranous submento-mentum which bears three pairs of setae. The terminal lobe (the syncoxite of Verhoeff(6)) is rounded and has a brown chitinous trident-shaped support (see Fig. 8). There are several pairs of setae and sensory tubercles between its arms. The single jointed palps are situated near the anterior border and each bears eight or nine small conical pegs at its apex.

Thorax and Abdomen. The lateral longitudinal folds are clearly seen dividing the body into epipleural, hypopleural and ventral regions.

The folds bear brownish setae of two sizes, arising from small tubercles, and the longer and shorter ones alternate. The body is clothed with very small evenly-spaced papillae.

The Thorax. On each side of the prothorax are four setae near the anterior, three near the posterior margin, two near the spiracle and two on the hypopleural lobe. On the meso- and meta-thorax there is one seta on the praescutum and five on the scutellum on each side. Laterally each epipleural and hypopleural lobe has one seta and each scutum has two lateral setae. The crural pads of the three thoracic segments each bear six small setae.

The Abdomen consists of ten segments but the tenth is very small and often withdrawn. The first eight abdominal tergites bear setae similar to those on the meso- and meta-thoracic tergites and each scutum has two lateral setae. On the epipleural lobes two setae are present just below the spiracle, and one on each hypopleural lobe.

The ninth abdominal segment bears a few small setae.

Spiracles (see Plate XVI, fig. 4). There are nine pairs of spiracles, one on the posterior edge of the prothoracic tergite and one on each of the first eight abdominal segments. These are situated in depressions just above the epipleural lobes and are hyaline with a slight yellowish tint. They are all nearly equal in size. Length about 58μ, width 35μ. They communicate with a two-lobed sac which bears characteristic internal ridges of chitin. The tracheal orifice is circular.

The abdominal spiracles lie parallel to the pleural folds with the tracheal openings in front; while the thoracic ones are at an angle of about 30° so that the tracheal openings are ventral to the sacs.

Pupation.

Just before pupation the larva straightens itself. The dorsal vessel becomes invisible. The ventral surface becomes flattened and the segments and wrinkles indistinct.

The Pupa (Fig. 9) is soft, pale cream in colour. The head is bent beneath the prothorax and carries no bristles. The thorax is the largest

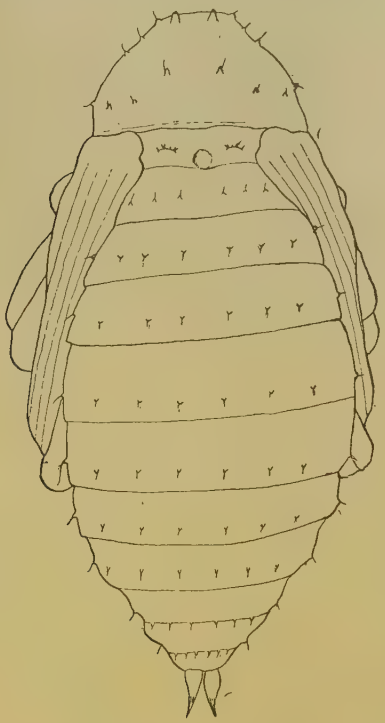


Fig. 9. Pupa of *A. cinctus*.

segment and its notum carries a number of short bristles each on a conical tubercle. The mesonotum has on either side of the centre a protuberance carrying a group of four bristles. In the middle line there is a round swelling near the base of the segment.

The metanotum carries three pairs of bristles each on a conical tubercle; and, about the centre, has a triangular depression wider in front.

The ninth abdominal segment bears a pair of stout caudal spines, much swollen at their bases. A small tenth abdominal segment is visible when viewed from the ventral side. On each of the first nine abdominal segments, four pairs of short bristles arising from small conical tubercles are visible from above. Length about 5 mm.

4. Distribution and Hosts.

This pest is widely distributed on the Continent, having been recorded from Holland, France, Germany, Sweden, Italy, Austria, Hungary and Russia. It is not, however, a very serious pest except occasionally in certain districts.

Dr L. Reh (Hamburg) (*in litt.*, 1924) says, "*Anthonomus cinctus* is almost unknown in Germany."

Dr P. Marchal (Paris) (*in litt.*, 1924) says, "*Anthonomus pyri*, Boh. is often found in France and some seasons does considerable damage in various fruit districts of this country. In general, however, its damage is not widespread. It is particularly troublesome in the valley of the Loire."

In France it is called "The winter worm" and also "The bud worm." In Germany it is called "The pear bud killer" or "The pear bud stinger." There its chief host appears to be the pear, as some observers do not mention it as an apple pest. According to Bedel (2) it is found in orchards and nurseries, and the larva lives in the buds of pear trees. Reitter (3) says, "In blossoming apples and pears." Decaux (7) says, "On pears mostly but found on apples in Picardy and near Paris." Ritzema Bos (8) obtained it from apple buds (in Holland) in 1915 and regards this as its first record from that source.

There is no record of this pest in this country up to 1921 when larvae were found in March damaging the buds of apples at Messrs Gardners, Wisbech, and from which adults were reared in June. Since then P. Harwood (1) records a single adult from Darenth Wood, Kent. Damage and specimens similar to the above were found at Messrs Gardners, Wisbech in 1922 and 1923.

In May, 1922 and again in 1923 we found larvae attacking the buds of apples in an orchard near Histon, Cambs. In 1922 Mr H. Goude (Horticultural Instructor for Norfolk) found specimens causing damage at Tilney in Norfolk.

On November 8th, 1922 we found several adults on "tanglefoot" bands on apple trees in an orchard near Cambridge, and on the same

trees we found eggs at the beginning of the following February and later larvae.

In December, 1922, Duffield records two specimens as being found in company with *A. pomorum* by Miss Worsley at East Malling, Kent.

Although this pest is found mainly on pear trees on the Continent, there is no record of it causing damage to, or being found on them in this country.

That this species has been found near Cambridge and Wisbech, in Norfolk, and at two places in Kent, points to the probability of its existence in this country for a number of years rather than to its recent introduction.

In spite of the fact that no collector has recorded its presence until recently (it is possible that it may be present in some collections as *A. ulmi*) it may have been present in small numbers and escaped observation. In support of this, we have been unable, even after careful search, to find this species during the winter 1923–4 on the trees near Cambridge from which we previously collected nearly fifty specimens.

This species is usually present together with *A. pomorum* and a few specimens might easily be overlooked amongst a large number of the latter.

5. Notes on Life-history.

In 1921 3rd stage larvae were found in hollowed-out buds on apple cordons at Wisbech. These buds were very noticeable as they had not increased in size, as had the normal buds. Several larvae were brought back to Cambridge and kept in the laboratory where they pupated before May 2nd. At the beginning of June they had all reached the adult stage.

In 1922 further damage by this weevil was found at Wisbech and in an orchard near Cambridge. Larvae were found as late as May 14th. On June 12th eleven beetles sent from Wisbech were "sleeved" on young apple trees and periodically examined in order to try and find out when egg-laying takes place. The "sleeves" were moved at intervals and the buds on the shoots where the weevils had been sleeved were carefully dissected. Several of the weevils died during the summer, but two of them lived until the end of October. No eggs were found.

The following February we began to search for the eggs on those trees where the pest had previously been found. On February 3rd, 1923 we found eggs in the buds of apple trees near those on which we had previously found adults. The presence of last year's hollowed-out buds

is useful in indicating which trees are likely to yield results in the search for eggs.

The buds which contain eggs are punctured by a small hole usually near the equator, but this is not always easy to see except by careful examination, owing to the dark colour of the outer bud-scales (see Plate XVI, fig. 3). Some buds were pierced in this way but contained no eggs.

The buds attacked are nearly always fruit buds, but we found two shoot buds also attacked.

Shoots with buds containing eggs were obtained from trees near Cambridge and kept under observation in the laboratory in order to determine the time spent in the various stages and also in rearing any parasites which might be present.

In the laboratory the first larva hatched on February 9th, *i.e.* six days after being brought in. On the trees the first larva was found on February 26th, and the last egg on March 16th.

The following table gives a summary of the life-history during 1923 as found in the orchard.

	When first found	When last found	Time taken in each stage
Egg	Feb. 3rd, but probably was laid much earlier	March 16th	?
1st stage	Feb. 26th	April 7th	3–3½ weeks
2nd stage	March 23rd	April 30th	2–3 weeks
3rd stage	April 6th	May 21st	3–4 weeks
Pupa	May 4th	June 18th	About 4 weeks

As soon as the larva hatches it begins to feed on the folded leaves of the bud. It lives through the larval stages in one bud, eating all the succulent portion and occasionally the softer portion of the stem below the bud. Reitter(3) says that the stem is hollowed out from 2–10 mm. below the buds, in attacks on pears in Germany. The damage which we found below the bud was never more than about 4 mm., and this only very rarely. A few of the infected buds partially open but they are soon killed if the larvae continue to feed. Occasionally small tortrix caterpillars may be found damaging the same buds.

6. Nature of Damage.

In the larval stages this pest kills the fruit buds and occasionally the lateral wood buds of the second year's growth of apple trees. We have never found terminal wood buds attacked, but in the cases we have investigated the percentage of buds killed has been very small. Fruit buds may be attacked when they are formed at the terminals.

The larva feeds on the succulent part of the buds and continues to feed until only the bud-scales remain, by which time it is ready to pupate. Each larva thus destroys one fruit bud.

Buds from which the weevils have escaped can easily be recognised as they are hollow, remain on the tree, and have a large lateral hole about 2 mm. in diameter.

In the adult stage (see Plate XVI, fig. 1) we have had little opportunity of studying the damage done by the adult in the orchard, but we have studied the injury they cause when "sleeved" on apple trees from June to February. In the "sleeves" during the summer the weevils caused the leaves to fall prematurely by puncturing the base of the petioles,



Fig. 10. Apple shoot showing the position of the punctures made by the adults of *A. cinctus.*

usually in several places. They also badly injured the young shoots by making holes in them, usually near a leaf, or through the bud-scars at the base of the shoot. A number of buds were found punctured, and also the succulent part of the fruit spurs below the buds.

After the leaves had fallen, they continued to feed by making holes in the buds: in this way a number of buds were killed, and others were punctured to their centres.

From the above it will be seen that this insect has great potentialities as a pest; but, up to the present, it has only been found in small numbers in this country. Near Cambridge over 90 per cent. of them were killed by external parasites. Near Wisbech no parasites have been found, but here the weevils have been kept down by continuous collecting.

It is important that collectors and growers who find this pest should record its presence, so that steps may be taken to try and prevent it from increasing in this country.

7. Parasites.

For the purpose of obtaining data on the life-history we collected and examined a number of damaged buds at frequent intervals. About the beginning of May we found a 3rd stage larva being attacked by an ectoparasitic larva, which we took to be *Pimpla pomorum*, and by the third week in May the percentage attacked by this parasite had increased enormously.

An examination of a number of buds on May 18th showed six larvae of *A. cinctus* all parasitised. On May 21st twelve larvae all parasitised were found and also one healthy pupa.

At the same time we examined a number of *Anthonomus pomorum* larvae and found that *Pimpla pomorum* were attacking less than 10 per cent. of these.

In order to determine the periods of the larval instars, we dissected the attacked buds. After dissection we put the parasites back with their host, but most of them died and only five pupated. (No cocoon was found.) Only one adult (a female) emerged, and the wings of this had to be removed and mounted to show their characteristics. This female was identified by Dr Waterston and by Mr G. L. R. Hancock as *Pimpla* (*Epiurus*) *pomorum*, Ratz. (with an unusually dark-coloured abdomen).

One larva (which was moulting) was preserved from the batch of *A. cinctus* parasites and in this we were unable to see any marked differences from the larva of *P. pomorum*, Ratz. as figured by Imms(9).

So thoroughly did this parasite do its work that we were unable to find specimens of *A. cinctus* during the winter 1923–4. Parasitism to this extent is unusual in this country and in this connection it should be borne in mind that the total number of the hosts (*A. cinctus*) was between 50 and 60 and more than half of these had been removed before the parasite appeared.

8. Control.

Up to the present we have had no opportunity of carrying out experiments on the best means of controlling this pest owing to the small numbers which we have found.

The control measures suggested by the Continental observers are the removal and destruction of the buds which are attacked in the spring



A B

Fig. 1.

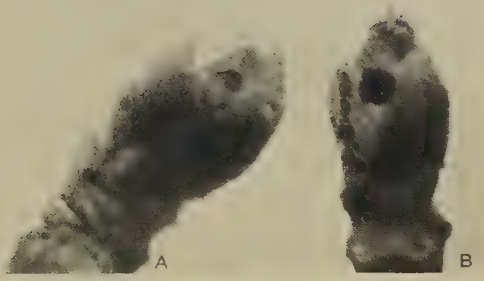


Fig. 3.

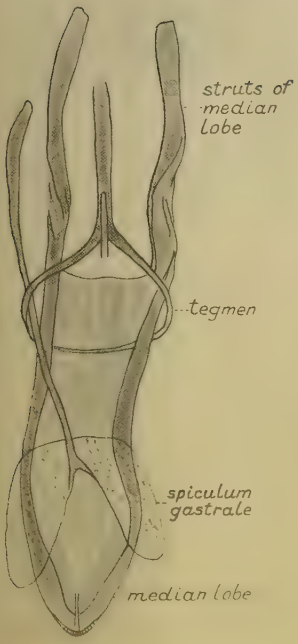


Fig. 2.



Fig. 4.

when the normal buds have opened, and at which time the infected buds are very noticeable.

The writers wish to express their thanks to Mr A. W. Rymer Roberts for his criticism and advice.

9. Summary.

Anthonomus cinctus is widely distributed on the Continent and occasionally, in certain districts, does serious damage to pear buds, and now and then is found injuring apples. In this country it is not yet recorded on pears, but was found killing apple buds at Wisbech, in 1921, and near Cambridge, in 1922. Adults have also been found in Kent.

The eggs are laid in apple buds (usually fruit buds) in the autumn, one egg only in each bud. The larva hatches at the end of February and eats out the succulent portion of the bud. It pupates in May and changes to the weevil stage in June. The weevils puncture the leaf stalks, buds and succulent portion of the shoots.

A description of the various stages is given.

A comparison is made between this species and those closely allied.

Near Cambridge *Pimpla pomorum* killed most of the larvae and pupae left on the trees after collecting.

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Explanation of Plate XVI.

Fig. 1. A. *A. pomorum.* B. *A. cinctus.*
Fig. 2. Male genitalia of *A. cinctus* (ventral view).
Fig. 3. A. Apple bud containing egg of *A. cinctus* photographed in March, 1923, showing the puncture made by the female before laying the egg. B. Apple bud showing the characteristic exit hole of the adult.
Fig. 4. Second abdominal spiracle of a third stage larva of *A. cinctus*, showing the outline of the chamber and its two sacs. ($\frac{1}{6}$″ objective used.)

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NOTES ON THE BIOLOGY OF *PSEUDOCOCCUS GAHANI*, GREEN

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Introduction

Pseudococcus gahani, a "Mealy Bug" indigenous to the British Isles, was first described by E. E. Green (1) on Red Ribes, and has since been recorded by him from *Ceanothus* sp., Laburnum, and potato tubers (2). This last record brings this species within the range of the Agricultural Zoologist, and the following observations were made on the material on which this record rests. Briefly the history of this insect as a potato pest is as follows. In December, 1922, Mr H. L. Jones, Horticultural Superintendent for Flintshire, discovered a "Mealy Bug" on seed potatoes at St Asaph, and this was reported by him to Mr C. L. Walton, the Adviser in Agricultural Zoology for North Wales. The potatoes were "King George," and had been placed during the previous July on the window ledge of a small empty greenhouse, but their place of origin and manner of infestation cannot be traced. Specimens of the coccid were sent to Mr E. E. Green, who identified them as *Pseudococcus gahani*, Green.

Subsequently all the infested tubers were removed to the laboratory at Bangor, where the life-history, habits, etc. of the coccid have been investigated. The tubers were very much shrivelled, and before work commenced it became necessary to transfer the stock of coccids to fresh tubers. For this purpose sprouted tubers of Sharp's Express and Great Scot were used. Detailed work was commenced in November, 1923.

Life-history.

Female.

(*a*) The adult female has already been described by Green (*op. cit.* (1)), and those examined agree with his, except for slightly greater length measurement. The average of ten specimens was, length 3·38 mm. and breadth 1·25 mm.

(*b*) Egg and ovisac. The egg is marbled, light brown and pale yellow, with a smooth unsculptured surface 0·38 mm. long and 0·25 mm. broad. It is elongate oval in shape and as many as 127 eggs are deposited in a single ovisac. This latter is usually elongate, about 4·5 mm. long and 1·5 mm. broad, and its shape depends to a large degree upon the surroundings. When formed on a smooth portion of a tuber it is roughly spherical, but when constructed in a groove or wrinkle of the skin, it is elongate. Whatever the shape, it is composed of white threads, closely felted together, which become brittle and ruptured with age.

(*c*) The first stage larva is brown, scantily covered with a white waxy secretion, which gives it a greyish colour. The eyes are situated at the base of the antennae, and are simple ocelli. The six-jointed antennae are 0·17 mm. in length. The inter-antennal margin of the body is lobed. Average length of the body 0·36 mm. and breadth 0·20 mm., length of leg 0·20 mm.

(*d*) In the second stage the antennae are 0·25 mm. long and the legs 0·50 mm. in length, while the body is 1·38 mm. long and 0·70 mm. broad. The inter-antennal margin is still lobed.

(*e*) The demarcation of the body into segments is more pronounced in the third stage, due to the greater quantity of white waxy secretion (except at the folds of the segments). The brown basal body colour becomes visible at these folds, producing a symmetrical pattern. The inter-antennal margin is uniform, there being no lobes as in stages 1 and 2. The loop of the mouth-filaments reaches as far as on a level with the bases of the hind pair of legs (the length of the latter being 0·50 mm.), and only a portion of the tarsi now project beyond the margin of the body. The average body length is 2·2 mm. and the breadth 1·1 mm.

Male.

(*a*) The adult male is a delicate, fragile insect with long antennae, legs and wings. The caudal extremity terminates in a pair of long, slender filaments, and a pair of shorter ones on the outer side. Body greyish-brown, covered with white powdery wax. The slender ten-jointed antennae are liberally covered with setae; the two basal joints, short and stout, the remainder elongate. Eyes in the form of large, simple ocelli are present on both surfaces of the head. In place of the mouth-parts, there is a plate of chitin. The wings are rounded on the outer side and hyaline in colour.

Length of body is 1·2 mm., breadth 0·35 mm.; length of leg 0·65 mm.; length of antennae 1·0 mm. Wing expanse 3·3 mm.

(*b*) The first stage larva is paler in colour than the female first stage larva. The body which is 0·45 mm. long and 0·30 mm. in breadth is covered with white waxy secretion. The inter-antennal margin is not lobed, and body segmentation is indistinct. The antennae are six-jointed, the basal joint stouter than the remainder and 0·25 mm. long. The mouth-parts are situated in front of the anterior pair of legs, and mouth-filaments reach almost as far as the posterior extremity.

(*c*) The second stage larva is of a brownish colour, the mid-dorsal and mid-ventral regions deeper than those at the sides. Length of the body 1·0 mm., breadth 0·50 mm.; length of antennae 0·37 mm., and the leg is 0·57 mm. long.

(*d*) In the pre-pupal stage the male is enclosed within a sac-like puparium, composed of a white, felted, thread-like, waxy substance, which is constructed from front to back, the head region being enclosed first. When extracted and mounted the colour of the insect is marbled, light brown predominating, but some of the white secretion still adheres giving it a greyish tint. Complete ecdysis takes place in this stage, and a lengthening of the body occurs. Wing-buds in the form of lobes develop on the body-margin between the bases of the first and second pairs of legs. Immediately prior to pupation the body length is 1·15 mm.; breadth 0·60 mm.; length of antennae 0·15 mm.; length of leg 0·25 mm. The abdomen is about one and a half times the length of head and thorax together.

(*e*) The brown-coloured body of the pupa is long (1·20 mm.) and narrow (0·42 mm.), with the general outline of the adult, the leg and antennal sheaths free. The antennal sheaths which reach as far as the second pair of legs are 0·42 mm. long. The narrow wing-sheaths, reaching beyond the last pair of legs, are 0·44 mm. long, pointed at the free end, and translucent, almost white in colour. The penial sheath in this stage is distinct and triangular. Length of leg 0·55 mm.

Life-cycle.

During warm, sunny days the females are very active, searching continually for their somewhat sluggish partners. Although close vigilance was kept, the writer failed to observe the two sexes pairing. At a mean temperature of 55° F. a female takes 7–9 days to construct the ovisac and lay her eggs. After completing the ovisac (in some instances two sacs are formed, but in such cases both are smaller in size), the female dies, remaining outside the structure, with the elevated posterior portion of the body resting on it. Larvae appeared six weeks after deposition

of eggs, the mean temperature during the period being 54·5° F.[1] These remained in the first stage for a month, but one batch remained for two months without moulting. The average period of the second stage is 4–5 weeks, and that of the third stage 6–8 weeks. The adult female attains full growth about a month after the final moult. Thus the whole life-period, from the time the eggs are laid to the construction of the ovisac by females of the new brood, is approximately six months.

Male larvae hatched during the first week in November, within a month after ova had been deposited. These larvae moulted on the 2nd and 3rd of December (mean temperature during the period 53° F.), remaining in the second stage for over a month. On January 5th, 1924, some of these larvae were observed to construct cocoon-like sacs, completing them in 40–48 hours. By opening these cases, and examining the males at definite periods, it was possible to observe the gradual change from the pre-pupal to the pupal stage. This took place in the majority of cases during the second week in February. On the 17th of March one pupa was found to cast the pupal skin, pushing it out at the anal extremity of the puparium. Two males hatched out two days later, and two others on March 26th. From these data it appears that the life-period of the male varies between 20 and 24 weeks.

HABITS.

On hatching from the eggs, the larvae are very active; the majority of them move towards light, and are capable of covering a distance of as much as 6 feet before becoming exhausted. Normally, after 12–18 hours activity they settle down upon the tubers, immediately inserting their long hair-like sucking apparatus into the tissues. When irritated the adult females exude a claret-coloured liquid in round drops, which soon dries resembling a small balloon, as already noted by Gahan (*op. cit.*(1)). The amount of honey-dew secreted by the females is small, only on rare occasions was it noticeable, even after prolonged infestation. Large batches of larvae hatched at various times in the laboratory, from the original material obtained, both before and after work commenced. In some instances great numbers of larvae were crawling about. The males are very sluggish in their habits, and none were seen on the wing. Only with difficulty could they be induced to move.

There seems little likelihood that this coccid will become a troublesome potato pest, for as far as the present observations go, there appears

[1] Ovisacs completed over eight weeks ago, and subjected to a mean temperature of 54° F., contain eggs which are still unhatched at the time of writing.

to be only two broods in a year. The life-period is also very extended, each brood taking on the average six months to complete the cycle. Potatoes are seldom stored or boxed for "seed" for more than six months at the most, and would need to be very heavily infested early on to suffer serious damage.

The work has been carried out in the laboratory of Agricultural Zoology at Bangor University, and I wish to thank Mr C. L. Walton, the Adviser in Agricultural Zoology for North Wales, for suggesting this work and placing the material at my disposal. I also wish to thank Mr E. E. Green for kindly identifying the specimens, for information and suggestions.

SUMMARY.

(1) *P. gahani* has previously been recorded by Green as occurring upon Red Ribes, Ceanothus, Laburnum and potato tubers. This last record is here elaborated.

(2) The female passes through five stages, while the male passes through six.

(3) The life-period in the two sexes is very extended, ranging between 20 and 24 weeks.

(4) Owing to the long life-period no fears should be entertained of this coccid becoming an economic pest.

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"FRUITINESS" IN WHEY

Part I. BACTERIOLOGICAL STUDIES

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The following investigation was undertaken to gain some knowledge of the origin of a characteristic "fruity" smell which sometimes develops in whey during the process of cheese making. The particular sample on which this work is based was procured at the Dairy Show of 1920, from whey which had developed a well marked odour.

Isolation of Causal Organism.

Since it was found possible to reproduce this fruity smell by means of direct subculturing from the sample of whey into sterile milk, an attempt was made to isolate the causal organism or organisms. Microscopical examination of the whey showed the presence of cocci, bacilli and yeasts. Tubes of sterile milk were inoculated with about 1 c.c. of the original sample of whey, and incubated at 22° C., 30° C., and 37° C. Each tube showed coagulation of the milk, but the tubes incubated at 37° C. alone showed fermentation and gave the characteristic sweet, apple-like odour. Microscopical examination of this culture showed the presence of numerous bacilli, yeasts and a few cocci. To effect the separation of these organisms, the milk culture was plated out on beer wort agar and whey agar. By repeated plating on beer wort agar, and finally by means of Barber's single cell culture method (2), pure strains of the yeast were obtained. The strain used in the experiments to be described below is specified as *YII*.

The isolation of the bacillus presented greater difficulty, since it did not grow readily on artificial media. The colonies on whey agar were very indefinite, appearing as a "haziness" in the medium. To the naked eye they suggested a flocculent precipitate in the medium, and it was only when examined under the low power of the microscope that they were revealed as masses of tangled threads of bacilli. These colonies readily curdled milk, but would rarely grow when transplanted on whey agar. In the process of isolation therefore it was necessary to pass this

organism frequently through milk. A single cell culture was eventually obtained by Barber's method (2), but this was discarded since it proved to be far less virile than the culture, obtained by repeated plating out, which was used in the experimental work recorded and is denoted in the text as *SCV*.

During the process of plating out, in addition to the yeast and the bacillus, strains of cocci and a species of mucor were isolated. A description of the two latter is not included in this paper since it was found that they were not concerned in the production of the fruity odour. This was produced only when the yeast and the bacillus were acting in conjunction in the milk.

Description of the Bacillus.

The bacillus grew well in sterile milk at temperatures from 30° C. to 37° C., with the formation of a firm curd. Examined under the microscope, the milk showed the presence of chains of bacilli, generally long and twisted, frequently capsulated. The bacilli were gram positive, and often showed the presence of granules when stained with methylene blue. Great difficulty was experienced in inducing growth on other artificial media. Whey agar was the only solid medium that gave satisfactory results, and even on this the organism required frequent passage through milk if its vitality was to be maintained. With repeated subculturing even into sterile milk it has at the present time lost much of its original vigour. Because of this aversion to artificial media fermentation tests carried out with litmus peptone water containing various carbohydrates and alcohols were without result. Further tests carried out with casein bouillon and yeast extract as the foundation of the media were rather more successful though still unsatisfactory. The results for these two series are shown in Table I, where the figures given represent the amount of acid, determined by titration with NaOH and calculated as lactic acid, produced in 100 c.c. of the medium.

Table I.

	Grams of Lactic Acid in 100 c.c. of Medium																
Medium	Glycerol	Xylose	Arabinose	Sorbitol	Mannitol	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
Casein Bouillon	0·05	0·11	0·14	0·06	0·06	0·18	0·26	0·18	0·11	0·07	0·11	0·18	0·035	0·05	0·05	0·05	0·05
Yeast Extract	0	0	0	0	0	0·18	0·59	0·20	0	0	0·09	0·50	0·07	0	0·2	0	0

The optimum growth occurred at about 35° C., though feeble growth could still be detected at temperatures as low as 20° C. and as high as 47° C. This bacillus produced in milk large quantities of inactive lactic acid (up to 2·5 grams of lactic acid per 100 c.c. of milk), together with a small quantity of acetic acid. There was also an increase in the amount of amino-nitrogen present.

This bacillus was one of the rod-shaped lactic acid bacteria. In many respects it closely resembled the thermobacteria (cf. the classification of Orla-Jensen (9)) commonly termed *B. bulgaricus*, a name which is generally used to denote a series of similar organisms. A comparison of the characters of the thermobacteria, and of the bacillus *SCV* isolated from the fruity whey, is shown in Table II.

Table II.

	Thermobacteria or *B. bulgaricus*	*SCV*
Growth on solid media	Difficult. Thin at surface	Difficult. Hazy growth
Morphology	Chains, often long and twisted	Chains, often long and twisted
Staining	Gram positive. Granules with methylene blue	Gram positive. Granules with methylene blue
Aerobiosis	Anaerobic conditions preferred	Anaerobic conditions preferred
Minimum temp. of growth	20°–22° C.	18°–21° C.
Optimum „ „	40° C.	About 35° C.
Maximum „ „	50°–52·5° C.	About 47° C.
Acid production ...	Levo, or more rarely, inactive, lactic acid (1·5–2·75 %). Acetic acid	Inactive lactic acid (2·5 %). Acetic acid
Fermentation reactions ...	Capricious	Variable
Alcohols and pentoses	No action	Very slight (cf. Table I)
Maltose and dextrin ...	Not usually, but if maltose, dextrin also	Both slight (cf. Table I)
Saccharose ...	No action	Very slight (cf. Table I)
Action on casein ...	Direct formation of mono-amino acids	Increase in amount of amino nitrogen in milk

The chief points of difference will be seen to be the lower optimum and maximum temperatures of growth of *SCV* and the slight variations in the fermentation tests.

Description of Yeast.

The yeast was a Saccharomycete. Spores were produced, though not abundantly, in two to three days at 22° C. The asci were elliptical and contained one to four spores. The vegetative cells reproduced themselves by budding, generally at both ends. The cells in a young culture

were typically oval, varying in size from $5{\cdot}5 \times 6{\cdot}4\,\mu$ to $9{\cdot}1 \times 10{\cdot}9\,\mu$, but round cells were also frequently present. In older cultures a great variety in size and shape was noted, a large proportion of the cells being sausage-shaped. These cells were much larger than the normal, and tended to hang together in chains. This tendency was most marked in cultures in beer wort which had been kept at a temperature of 22° C. This yeast was able to grow in beer wort from temperatures of 3° C. to 45° C., with fermentation and formation of scum from 11° C. to 45° C. This scum first developed as a ring at the surface, round the sides of the tube, and later formed a waxy covering over the surface. On agitation it was readily broken and sank to the bottom of the tube. The formation of the scum was most rapid at temperatures between 22° C. and 30° C., when it made its first appearance in seven to nine days, and completely covered the surface in about fourteen days. It consisted largely of chains of sausage-shaped cells showing a marked tendency to mycelium formation. This tendency was much less at temperatures above and below these limits. At very low temperatures there was neither scum formation nor fermentation and the cells were generally pear- or club-shaped and showed little tendency to hang together in colonies.

On beer wort agar it produced a heavy cream-coloured growth which later became brown and somewhat "granular" in appearance. Colonies on beer wort agar were cream-coloured, opaque and hemispherical. In stab cultures in beer wort agar and sugar gelatine, there was growth throughout the stab and also good growth on the surface. In one instance in which such a stab culture in sugar gelatine was kept for three months in a cold room, the growth on the surface was found to consist of cup-like erections composed of chains of long irregular cells and small round ones in groups. In the depth of the medium the growth appeared as a succession of discs with feathery edges. These consisted of chains (often branching) of elongated cells, with groups of smaller cells, showing on the whole a marked resemblance to a mycelium of *Monilia*.

The yeast was able to grow in milk, but caused no apparent change. Fermentation occurred in solutions containing saccharose, glucose, fructose and galactose, but not in lactose.

Since this yeast forms spores it must be classed among the *Saccharomycetes*, and since it reproduces itself by budding, and sets up alcoholic fermentation in sugar solutions, it must be placed in the genus *Saccharomyces*. In this genus it belongs to the sub-group which ferments dextrose, maltose and saccharose, but not lactose, a sub-group which includes

most of the yeasts used in the making of wines and beers. Amongst these it most closely resembles *S. ellipsoideus*, but differs markedly from it in that it does not readily form spores.

As the work progressed it became clear that the fruity odour developed only when the bacillus and the yeast were both present in the milk. It also became evident that the bacillus was capable of fermenting lactose, but the yeast, although it readily fermented glucose and galactose, was without action upon lactose. Hence it seemed reasonable to suppose that the bacillus functioned by converting the lactose into simpler sugars, which were then available for fermentation by the yeast with the production of the characteristic odour. A study of the chemistry of the reactions concerned in the production of the fruity whey is included in Part II.

Part II. CHEMICAL STUDIES

By E. R. HISCOX, M.Sc.

Since the production of the fruity odour was apparently due to the combined action of the bacillus *SCV* and the yeast *YII* on the milk (Part I), it was thought to be of interest to identify the substance or substances to which the characteristic odour was due, and to study the chemical reactions involved.

The bacillus was inoculated into sterile milk and allowed to grow until a clear whey could be separated from the curd. Tests carried out on this whey confirmed the view that the acid produced was mainly, if not wholly, lactic acid.

A mixed culture of the bacillus and the yeast was next allowed to grow in raw and sterile milk and whey. The characteristic odour readily developed and when the products were distilled in steam, the vapours being collected in water contained in ice-cooled receivers, the distillates possessed in a more or less marked degree the characteristic odour sought, and gave indications of the presence of an aldehyde, ethyl alcohol, and a trace of acidity.

A series of more detailed analyses of cultures in sterile milk[1] of the bacillus, the yeast, and the bacillus and yeast combined, was undertaken

[1] The milk used in these experiments was sterilised by heating in the autoclave at 115° C. for 15 mins. The sterility of the milk was tested by subsequent incubation at 30° C. for not less than 10 days.

in order to study their action, singly and jointly, on the milk. The analyses were carried out in the following way:

The titratable acidity. This was determined by titration with $N/4$ NaOH solution and estimated as lactic acid.

Sugars. The sugar was estimated as lactose, by means of Fehling's solution, after precipitation of the protein with $CuSO_4$ and KOH (Ritthausen (5)). In order to determine whether the sugar was purely lactose, or whether glucose and galactose were also present, the sugar in a further sample was hydrolysed by heating for 30 minutes at 115° C. with 4 per cent. H_2SO_4. The sugar in the neutralised solution was determined as above. For pure lactose the reducing power is increased by 40–43 per cent. (8) by hydrolysis. A lower percentage denotes the presence of simpler reducing sugars.

The volatile acidity was estimated by steam distilling a portion of the milk culture, and titrating the distillate with $N/4$ NaOH solution. The nature of the volatile acid was determined with the aid of Duclaux' numbers (4). In this case it proved to be practically pure acetic acid.

Alcohol. The alcohol was detected by means of the iodoform test and Klöcker's modification of Pasteur's drop reaction (6). In those cases in which quantitative determinations have been made, the steam distillate was re-distilled into a pyknometer, and the amount of alcohol estimated from the specific gravity of this liquid.

Aldehyde in the distillate was detected by its peculiar odour, and by the yellow colour and characteristic smell of aldehyde-resin which developed on boiling with KOH. In the later stages of the work the delicate and practically specific test for acetaldehyde, by means of sodium nitroprusside and piperdine (Rimin's reaction—Lewin's modification (10)), was almost exclusively used. Quantitative determinations were made by shaking the distillate with a solution of sodium bisulphite and estimating the residue of bisulphite solution by titration with standard iodine solution.

First Series.

Flasks containing 500 c.c. of sterile milk were inoculated with the bacillus, the yeast, and a mixture of the bacillus and yeast, respectively. Parallel cultures were made in sterile milk containing an excess of $CaCO_3$. The cultures were incubated at 30° C. for five days, and then stored in a dark cupboard at room temperature until analysed—5 to 10 days in the first case, 20 to 30 days in the second. During the period of incubation the flasks containing the $CaCO_3$ were frequently shaken to prevent the

accumulation of acid. In these circumstances it was not possible to determine the titratable acidity, and it was necessary to add H_2SO_4 to the milk to liberate the volatile acid before the distillation in steam.

The results of the analyses are given in Table III:

Table III.

	Culture	Total titratable acidity as lactic acid gms. / 100 c.c. milk	Volatile acidity as acetic acid gms. / 100 c.c. milk	Sugars as lactose gms. / 100 c.c. milk	Increase in reducing power after hydrolysis %	Alcohol	Aldehyde
1	Milk – Control	0·204	–	5·400			
2	Milk + *YII*	0·214	–	5·218		–	–
3	Milk + *SCV*	2·54	0·04578	3·074	25·5	–	–
4	Milk + *SCV* + *YII* ...	2·347	0·03993	0·984		+	+
5	Milk + $CaCO_3$ – Control ...		0·0079	5·212	38·7	–	–
6	Milk + $CaCO_3$ + *YII* ...		0·0113	5·134	36	–	–
7	Milk + $CaCO_3$ + *SCV* ...		0·1092	0	–	–	–
8	Milk + $CaCO_3$ + *SCV* + *YII*		0·1094	0	–	+	+

From this table it will be seen that the yeast, whilst able to grow in milk, had no apparent action upon it, though probably a very small quantity of lactose was used for purposes of assimilation. The bacillus, on the other hand, had a considerable action. A large quantity of lactic acid was produced together with a small quantity of acetic acid. Almost half the sugar was consumed, and since the increase in the power of reduction after hydrolysis was 25·5 per cent., it may be assumed that the lactose was converted into glucose and galactose by the action of the bacillus. When the bacillus and the yeast were both present a very active fermentation took place with the production of alcohol and aldehyde. The two organisms acting in conjunction were able to remove practically the whole of the sugar even when $CaCO_3$ was not present. The small residue may have been embedded in the hard lumps in the curd, and thus would not have been available to either organism.

Second Series.

It was thought that probably the amount of aldehyde produced could be increased if the conditions were made more favourable for the action of the yeast, *e.g.* by the addition of glucose to the milk. Table IV gives the record of the results of the analyses of a series of cultures of the organisms in milk and milk to which glucose had been added, undertaken primarily to compare the aldehyde production under these conditions.

In this table an attempt has been made to denote the sugar as quantities of lactose and glucose. This has been done by assuming that the increase in reducing power after hydrolysis is exactly 40 per cent. for lactose. The figures given are, therefore, only approximate. The high percentage increase in No. 5 was probably due to the fact that the glucose used was "Commercial" and contained dextrin.

Table IV.

	Culture	Total titratable acidity as lactic acid gms. / 100 c.c. milk	Volatile acidity as acetic acid gms. / 100 c.c. milk	Sugars: Lactose gms. / 100 c.c. milk	Sugars: Glucose gms. / 100 c.c. milk	Increase in reducing power after hydrolysis %	Alcohol gms. / 100 c.c. milk	Aldehyde gms. / 100 c.c. milk
1	Milk – Control	0·204	–	5·214	–	41·5	–	–
2	Milk + *SCV*	2·41	0·03828	1·374	0·798	25·8	–	–
3	Milk + *SCV* + *YII* ...	2·100	0·0245	0·502	–	77·4	+	0·1118
4	Milk + glucose – Control ...	0·259	0·0184	5·214	3·124	19·6	–	–
5	Milk + glucose + *YII* ...	0·349	0·0175	5·428	–	53·3	1·34	Trace
6	Milk + glucose + *SCV* + *YII*	2·57	0·0513	2·00	–	87·0	+	0·1720

From the table it is seen that there was a slight increase in the amount of aldehyde produced when glucose was added to the milk, although the sugar was not entirely consumed. The vapours which passed over at the beginning of the distillation had an unmistakable odour of acetaldehyde.

The yeast fermented the whole of the glucose with almost quantitative production of alcohol, leaving the lactose practically untouched.

The results of these analyses confirm the hypothesis that the bacillus functioned by converting the lactose into sugars which were available for fermentation by the yeast. The fruity odour was due to the products of this fermentation, namely, acetaldehyde and alcohol, admixed with acetic acid produced by the action of the bacillus.

Third Series.

Further experiments were carried out to test whether the presence of both organisms in the living state was essential for the production of the characteristic fruity odour. The bacillus was inoculated into sterile milk and allowed to grow. At the end of about a month the culture was subjected to a temperature of 100° C. for 30 minutes. Inoculations were made from this heated culture into milk in order to ascertain that the bacillus had been killed. A further sample was titrated and distilled in steam. The distillate showed the presence of volatile acid, but no

trace of alcohol or aldehyde. The residue of the milk culture was inoculated with the yeast, and incubated at 30° C. The characteristic smell developed, and when the culture was analysed, the titratable and volatile acidities of the milk were found to be practically unchanged, but alcohol and acetaldehyde were present in the distillate.

Analyses of cultures of the yeast in milk + lactic acid + glucose; milk + acetic acid + glucose; milk + glucose; peptone water + glucose; water + glucose, readily demonstrated the presence of alcohol and acetaldehyde in each case, although there was only a trace in the culture in water + glucose, probably due to scanty growth and fermentation. Thus it would seem that the glucose was directly concerned in the production of the acetaldehyde.

Neuberg (7) has recently shown that acetaldehyde is formed as an intermediate product in the degradation of glucose during alcoholic fermentation, and that it can be retained and detected in the solution if $NaHSO_3$ has been added. More recently Abderhalden (1) has demonstrated that it can be adsorbed by certain colloids present in the medium. On the other hand, acetaldehyde may be formed by the direct oxidisation of ethyl alcohol.

In order to test which, if either, of these reactions was responsible for the production of the acetaldehyde, two flasks of sterile milk were inoculated with the yeast and the bacillus. One was placed in a jar under anaerobic conditions (coal gas—exhaust—pyrogallol), the other under ordinary aerobic conditions. Both flasks were incubated at 37° C. for three days, by the end of which time the milk had coagulated and a vigorous fermentation set in. They were then removed to an incubator at 14°–16° C., and at the end of 14 days the contents of both flasks were distilled in steam. Each distillate contained ethyl alcohol, showing that alcoholic fermentation had taken place, but in that from the culture which had been grown under anaerobic conditions no trace of acetaldehyde could be found although the distillate from the aerobic culture gave a definite reaction.

The theory that the production of the acetaldehyde was the result of oxidisation of the alcohol was confirmed by placing the yeast in 1 per cent. alcohol solution. Under ordinary aerobic conditions, at a temperature of 14°–16° C. acetaldehyde could be detected in the solution within a few hours. Under anaerobic conditions (as described above), no acetaldehyde could be detected at the end of 14 days, but when this sample was returned to the incubator under aerobic conditions, acetaldehyde was formed within 24 hours.

It was thought of interest to study the interaction of the yeast and other lactic acid bacteria commonly present in milk and milk products. *B. bulgaricus*, which is known to hydrolyse lactose (3), gave, in combination with the yeast, a vigorous fermentation, with good production of acetaldehyde. Combinations of two strains of "starter" bacteria and the yeast, and of *B. coli* and the yeast, gave negative results.

Of six unidentified, different strains of yeast tested for their action upon 1 per cent. alcohol, four readily produced acetaldehyde. One of these, a lactose fermenting yeast isolated from a "blown" cheese, produced acetaldehyde when inoculated into sterile milk.

By the courtesy of Mr Todd, a number of cheese were made at the British Dairy Institute under the supervision of Mr Todd and Mr Holmes, from milk which had been inoculated with these organisms, in order to test their influence on the market value of the cheese. The milk used was of low bacterial content, and all utensils were carefully steamed before use. The quantity of milk and the amount of space available at the time were limited. The type of cheese chosen was therefore the Smallholder, which is quickly made and is small in bulk. Moreover, the curd is scalded at a high temperature, and is comparatively slightly pressed, conditions which should be favourable to the growth of the bacillus and subsequent action of the yeast, respectively. On the other hand the short time during which the high temperature was maintained tended to limit the growth and consequent action of the bacillus.

The bacillus *SCV* originally isolated from the fruity whey had at the time become very slow in its action. A vigorous culture of *B. bulgaricus*, which hydrolysed lactose and brought about the same reaction in combination with the yeast, was therefore substituted for it in these experiments. In two batches of cheese *B. bulgaricus* was used as a starter, in a third a mixture of *B. bulgaricus* and a pure culture of a "starter," and in the fourth an ordinary dairy "starter" only.

The yeast was added to the milk after it had been placed in the vats. Control cheese were made in each case without the addition of the yeast. During the making of the cheese a distinct fruity odour was noted in the vats containing *B. bulgaricus* and the yeast, particularly after the cutting of the curd. In all other cases it was entirely absent. When the cheese were ripened, those containing *B. bulgaricus* and the yeast had developed a pronounced fruity flavour, and alcohol and acetaldehyde could be detected in the steam distillates from these cheese. The cheese containing the ordinary dairy starter and the yeast was very slightly "yeasty," but the characteristic fruitiness was not present, neither could

acetaldehyde be detected in the steam distillate. The control cheese were normal in flavour.

Summary.

1. A case of "fruitiness" in whey was found to be due to the interaction of a bacillus and a yeast.

2. The bacillus, during fermentation, hydrolysed the lactose with the production of glucose and galactose.

3. The yeast was without action on lactose, but readily brought about alcoholic fermentation of the products of the bacterial hydrolysis.

4. The fruity odour was due to acetaldehyde admixed with alcohol and acetic acid.

5. The acetaldehyde was produced by oxidisation of the ethyl alcohol by the yeast.

6. The "fruitiness" was reproduced in cheese containing *B. bulgaricus* and the yeast.

This work was carried out at the National Institute for research in Dairying, and at the Royal Technical College, Copenhagen. I wish to express my thanks to Professor S. Orla-Jensen for much valuable help and encouragement, and to Professor Søncke Knudsen for his kind interest and valuable suggestions.

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REVIEWS

Colour-changes in Animals. The Pigmentary Effector System: A Review of the Physiology of Colour Response. By Dr Lancelot Hogben. (Biological Monographs and Manuals. Pp. xi + 152. Edinburgh and London: Oliver and Boyd, 1924. 10*s*. 6*d*. net.)

Whilst every student of zoology and many physiologists will be grateful to the author for this attractive volume on the physiology of colour-response in Vertebrates and Crustacea, it would be a mistake to imagine that Dr Hogben treats generally of the biology of animal pigmentation. He has selected a definite problem—the factors that determine alteration in the mobile pigment-cells that give colour to the skin—and leaves untouched the wider problems of animal chromatology. The cells that exhibit pigmentary expansion and contraction are accepted as mechanisms responsive to stimulation and are regarded as forming one of the trinity of "effector systems" along with muscle and cilia. We are not told whence they come in development nor is the biochemistry or the physiology of pigmentation discussed. Dr Hogben has no doubt done the right thing by confining this book to the restricted problem but we hope that the editors of the series of which this volume is the first will provide another dealing with the wider aspects of that pigmentary system whose regulation is here described by one who has taken an active part in its investigation.

The pioneer in the work that has led up to our present knowledge of radiation and retraction of mobile pigment was Lord Lister, who with true prophetic insight drew attention in 1858 to the value of the cutaneous pigmentary system of the frog not only as affording insight into the biology of animal colour-change but as giving test-objects for investigation of pharmacological effects, thereby anticipating by some sixty years the modern use of chromatophores as indicators of the action of drugs. The idea of the term "effector system" as applied to the variable pigmentary system may indeed be traced to Lister's famous paper where he says "the pigmentary system also promises to render good service to toxicological enquiry. Hitherto in experiments performed on animals for that object, attention has been directed chiefly, if not exclusively, to the effects produced upon the action of the nervous centres, the nerves and the muscles. In the pigment-cells we have a form of tissue with entirely

new functions which though apparently allied to the most recondite phenomena yet produce very obvious effects...and the effects produced are so obviously indicated by the changes in colour of the integument that I venture to recommend this method of investigation to those who are occupied in studying the action of poisons."

The development of endocrinology eventually led to the adoption of Lister's suggestion in a modified form. Adrenalin was shown to produce general contraction of the dark pigment in the skin of frog by Corona and Moroni in 1898, and removal of the pituitary was seen to be followed by extreme pallor in the experiments on tadpoles made by various physiologists between 1914 and 1919. But it was only in 1921 that Hogben and Harley in England and Swingl in America performed the crucial experiment of testing the effects of pituitary extract on the larvae of newts and of the frog and showed that when taken from the posterior lobe of this organ (of any vertebrate) it causes maximum darkening of the skin of frogs. This rapid and general reaction naturally raised doubts as to the accepted view that the pigmentary system in these animals is controlled by the nervous system. The complexity of the nervous control of the vascular system—that system through which the pituitary hormone exercises its influence upon the pigment-cells—was sufficient, when fully realised, to call for the most careful analysis of any colour-effects that were associated with nervous stimulation in the frog, especially in view of the fact that no indubitable nerve-endings in these pigment-cells had ever been demonstrated. Doubts of the reality of any effective nervous control have hardened as more work has been done on the Amphibia and the conclusion reached by Dr Hogben is that the colour-changes of these animals are mainly, if not exclusively, responses to a pituitary hormone the secretion of which fluctuates in correspondence with the action of natural stimuli. Whilst recognising that this correspondence involves an unsolved mystery, the colour-changes of Amphibia may be regarded as hormonic.

The case of fishes shows how dangerous it is to generalise from the phenomena offered by a single order of animals. The colour-changes of certain Teleosts and of Dipnoi offer some of the most rapid and striking cases in the whole animal kingdom and it would have added to the interest of Dr Hogben's work if an account of these colour-effects as seen under natural conditions had been given. The evidence taken from experimental work is however fully discussed and reveals many interesting points that differentiate the chromatophores of fishes from those of Amphibia. Direct evidence for nerve-endings and nervous control has been obtained

by Ballowitz and Ponchet. The preparations made by Ballowitz were seen by the reviewer in 1893 and were even more convincing than the excellent figures of his classical paper. But the control of colour-response in fish is not nervous only. Some evidence is forthcoming that as in Amphibia internal secretion plays an important part in the initiation and regulation of the display but in an opposite sense. Pituitary extract, for example, induces contraction and consequently pallor and not expansion or darkening of the skin as is the case in Amphibia. The predominant controlling factor in fish, however, appears to be direct sympathetic nervous action.

The case of reptiles shows a third class of controlling influences; adrenal secretion. The chapter in which Dr Hogben discusses the reactivity of Reptiles involves a previous knowledge of Langley's work on the autonomic nervous system that most of his readers will not possess and the terms used for the action of drugs might have been explained but the general results are (as is always the case with investigations on this little-known order) not very clear. Light may have a direct action on the chromatophores of the skin. Excitement (through adrenal secretion) produces pallor. Pituitary extract appears to have no effect. This section on Reptilian colour-change is a valuable *résumé* and the only omission we have noticed is that Schmidt's important monograph though quoted is not given in the list of literature.

The concluding chapter gives a critical account of the colour-responses exhibited by Crustacea. To one who has taken part in this work, it is of the greatest interest to find comparative physiology coming into its own at last and bringing to bear upon the problems of coloration in *Hippolyte* the new ideas and new methods of interpretation that have been gained mainly in the pursuit of Vertebrate physiology. These prawns appear to live in closer relation with their external environment, whereas the metachromatic Vertebrates are greater humorists. Their chromatophores possess a direct basal reactivity to light and darkness and also an indirect nervous reactivity determined by the direction in which light enters the eye after leaving the background. The former basal control has become fixed as part of the periodic daily rhythm—night phase of activity and day phase of rest—that is exhibited by the whole organism and all its organs. Whether in addition to this, there is also in these prawns an internal humoral regulation analogous to that of Vertebrates, we do not know, but if it exists it appears to play a negligible part in the control of the pigmentary system. More important still is the question of the relation of the chromatophores of Crustacea to

nutrition. Since Gamble and Keeble published their evidence on this subject in 1906, the fat-soluble vitamin problem has arisen and the attention of investigators has been mainly directed to the case of Vertebrates and especially of Rats. In *Hippolyte* there is a very suitable object for testing the significance of mobile lipochrome pigment in its relation both to the presence of fat in light and to the absence of fat in darkness. The hypothesis tentatively advanced in 1906 of a photosynthetic action of lipochrome pigment in these animals is, we are glad to see, referred to in this work and it would have been an advantage to future workers in this unexplored field, if this rather crude hypothesis had been considered in the light of the economics of fat-soluble vitamins especially as *Hippolyte* ranges into the Arctic seas.

This volume is a sign of the *rapprochement* between zoology and physiology and forms the first of a series that will be welcomed by all biologists. It contains a finely critical appreciation of the factors governing colour-response and shows not only what is known but what the next problems are and how they may be approached. The author, the editors and the publishers are to be congratulated on the successful launching of such a timely aid to biological research.

F. W. GAMBLE.

Outlines of Fungi and Plant Diseases for Students and Practitioners of Agriculture and Horticulture. By F. T. Bennet. Macmillan & Co. 1924. *7s. 6d.*

This volume consists of a series of extended lectures with practical exercises, is written in an old-fashioned style and is not particularly up-to-date. Part I consisting of sixty-seven pages purports to be an "introduction to the study of Fungi in general" but is distinctly lopsided, for whilst Lichens have ten pages devoted to them, the Rusts and Smuts are not even mentioned. Furthermore it contains much to cavil at. "A structure consisting of living cells only" is not an adequate definition of a thallus: *Pythium de Baryanum* is by no means so common as suggested; to speak of the "*species*" *Penicillium glaucum* is misleading as is also to describe yeast as an anaerobic organism; whilst the author's views on fungus phylogeny need widening. Part II which deals with plant diseases and the fungi which cause them is perhaps better than Part I but is equally in need of critical revision. The inverse relation between vigour of host and incidence of disease is by no means so clear as described nor can much weight be given to the argument that

"Cultivated plants are of more delicate constitution" than wild plants. There is at the time of writing no published evidence of the very important statement on page eighty-six regarding the infection of immune potatoes by *Synchytrium endobioticum*, and it is still premature to class Virus Diseases along with diseases of known bacterial origin. Many of the illustrations might be improved whilst the method followed in referring students to literature is incomprehensible. The volume is, however, a brave attempt to fill a noteworthy gap in English botanical literature and should a second edition be called for a drastic revision would produce a very useful book.

The Evolution of Plants. By Duckenfield Henry Scott. Home University Library. Williams & Norgate, 1924. 2*s.* 6*d.*

A reprint of Dr Scott's rather wonderful little book first published in 1911 and now re-issued at, alas! two and a half times its original price. The volume is unique in its breadth of view and clarity of vision and is still quite the best introduction to the study of the evolution of plants. It seems a pity therefore that Dr Scott should not have produced a new edition incorporating the main results and points of view arising from the work of the past decade. Fortunately these researches can be found put into fine and simple perspective in Dr Scott's *Extinct Plants and Problems of Evolution* published recently.

An Introduction to the Study of Heredity. By E. W. MacBride. Home University Library. Williams & Norgate, 1924. 2*s.* 6*d.*

In the popular view the study of heredity has come to be synonymous with Mendelian investigation and Professor MacBride has done good service in writing the present interesting volume putting the subject in a truer perspective. The book is a vigorous case for Lamarckism and a criticism of the efficacy of natural selection as the originator of new species and of any great light to be thrown on the problem of evolution by Mendelism. The chapters are somewhat unequal but that on the inheritance of sex is a masterly summary. Professor MacBride's appreciation of Tornier's researches is interesting but it is doubtful if their better knowledge will prove quite so revolutionary as the author thinks. The last chapter deals with the laws of heredity on human affairs and this is the weakest portion of the book. The author is a "race dogmatist" and accepts crude ideas of racial psychology perhaps a little too easily—his description of the shuddering Nordic taxpayer is distressing.

The Biological Foundations of Society. By Arthur Dendy. Constable & Co. 1924. 7*s.* 6*d.*

Professor Dendy's book is the outcome of a series of lectures delivered at King's College and if the lectures were followed by discussions one can only regret whole-heartedly that one was not present. The work is largely an account of the structure and organisation of the living unit, its hereditary and environmental relationships and its social evolution. Interwoven with the strictly biological argument is a running commentary of sociological observation which one will think foolish or wise according to one's political or social opinions. So closely are the biological facts and sociological hypotheses interwoven that many readers may feel that the latter have taken the reins into their own hands and tended to guide the interpretation of the former. Thus Professor Dendy's naïve psychology, his by no means progressive ideas of the relation of Capital and Labour, Communism and the House of Lords, Birth control and Slum populations, tend to run away with him and lead him into somewhat questionable analogic chains of reasoning. All this is, of course, helped by the author's Lamarckian views which are very apparent throughout. Here and there it is a little difficult to take Professor Dendy quite seriously, as, for example, when he quotes apparently with approval Gates' account of the girl whose ancestors had worn ear-rings and who was born with perforated ear-lobes! On human racial matters Professor Dendy speaks perhaps a little more dogmatically than the evidence warrants, and many of his views on social and national problems are open to criticism. Professor Dendy has, however, written a very interesting book on an aspect of Applied Biology far too neglected by Biologists, and, that one differs from him in perhaps most of his points of view and applications, is only evidence of the book's stimulating quality.

WM. B. BRIERLEY.

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